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The Effect of Ozonation of Organics in Wastewater

Colorado Univ. at Denver

Prepared for

Health Effects Research Lab.
Cincinnati, OH

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The effect of ozone treatment of domestic wastewater and various model compounds has been determined with respect to trace organic components. Organic constituents were identified in wastewater that was treated with ozone at the Upper Thompson Sanitation District Treatment Plant, Estes Park, Colorado. The primary products of ozonation of this wastewater are n-alkanes and n-aldehydes. Additional wastewater from this and other sources, including paper mills and chemical manufacturing facilities, were ozonated under laboratory conditions to determine the chemical effect of ozone treatment. Laboratory studies were made of ozonation of several model compounds, including caffeine, oleic acid, saturated aldehydes, dicyclopentadiene, diisopropylmethylphosphonate, and p-chlorophenylmethyl sulfone and reaction products determined.

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THE EFFECT OF OZONATION OF ORGANICS IN WASTEWATER

by

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Grant No. R 804472

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, and biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

This report describes a study of ozone disinfection of domestic wastewater and ozone treatment of several model compounds. With a better understanding of reaction products, a better assessment can be made of the qualities of ozonation compared to other disinfection processes, such as chlorination.

R. O. Garner
Director
Health Effects Research Laboratory

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ABSTRACT

The effect of ozone treatment of domestic wastewater and various model compounds has been determined with respect to trace organic components. Organic constituents were identified in wastewater that was treated with ozone at the Upper Thompson Sanitation District Treatment Plant, Estes Park, Colorado. The primary products of ozonation of this wastewater are *n*-alkanes and *n*-aldehydes. Additional wastewater from this and other sources, including paper mills and chemical manufacturing facilities, were ozonated under laboratory conditions to determine the chemical effect of ozone treatment. Laboratory studies were made of ozonation of several model compounds, including caffeine, oleic acid, saturated aldehydes, dicyclopentadiene, diisopropylmethylphosphonate, and p-chlorophenylmethyl sulfone and reaction products determined.

This report was submitted in fulfillment of Grant No. R-804472 by the University of Colorado, under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period May 1, 1976 to April 30, 1979 and work was completed as of May 31, 1979.

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SECTION 1

INTRODUCTION

Recently there has been an increasing awareness of and growing concern about the large number of organic compounds in both industrial and domestic wastewaters. Because many of these effluents eventually enter surface and/or groundwater systems, they have the potential to impact on environmental quality and human health. This concern was heightened in recent years by the discovery that chlorine, which is commonly used as a disinfectant in water, can increase the concentrations of some toxic organic compounds such as chloroform. This discovery has led to consideration of alternative methods of water treatment which would reduce the amounts of potentially harmful organic chemicals entering potable water supplies. These alternative disinfectants include ozone, ultraviolet radiation, chlorine dioxide, and bromine chloride.

The study described in this report was undertaken as a partial evaluation of the chemistry of ozone when used as a disinfectant in water. The thrust of the project was basically two-fold. In one part, an investigation was conducted at the 1.5 mgd wastewater treatment plant at Estes Park, Colorado. This plant, which uses ozone to disinfect its effluent, is operated by the Upper Thompson Sanitation District (UTSD). During the period of this grant an evaluation of the performance of the plant was being carried out by M & I Consulting Engineers, Inc. with partial funding from EPA. In addition to the evaluation of this treatment plant, studies of the effluents from various industries were carried out. These industries included paper mills and chemical manufacturers. In the second part, various model compounds were ozonated under laboratory conditions to determine the ozonation chemistry of specific compounds.

The objectives of the research, with respect to the UTSD facility, were to:

- Determine what organics are present in the UTSD plant influent.
- Determine what organics are removed by ozonation.
- Determine what organics are formed by ozonation.

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- Determine what organics are unchanged by ozonation.

- Determine what parameters affect chemical oxidation by ozone.

Additional studies were made with the following objectives:

- Identification of important components in domestic and industrial wastewater untreated by ozone.

- Determination of relatively long-lived intermediates produced in reaction from ozone and organics.

- Identification of stable reaction end-products.

- Investigation of the mechanism of product and intermediate formation from ozone and organics.

- Investigation of the halide ions and possible indirect chlorination of organics found in wastewater following treatment with ozone.

Although ozone treatment of water and wastewater is used in many European systems, only a few instances of ozone disinfection are found in the U.S. An overview of ozone treatment of water is found in the volume edited by Evans[1] and the Proceedings[2] sponsored by the International Ozone Institute. Miller and Rice[3] have given a brief overview of ozone treatment systems. A state-of-the-art review of several disinfection alternatives, including ozone treatment has been prepared by the U.S. Environmental Protection Agency.[4] Bailey[5] has published the first of a series of volumes reviewing ozone chemistry.

General mechanisms for ozone treatment of water have been prepared by Hoigne and Bader[6] and Hewes and Davison.[7] Kuo, et al,[8] have identified products of aqueous ozonolysis of 2-proparol, acetic acid and oxalic acid. Eisenhauer[9,10] has studied the reaction of phenol with ozone as have Gould and Weber.[11] Pryde, et al,[12] have studied the ozonation of 1-decene and methyloleate in water. Several papers have appeared as a result of the studies described in this report.[13-17]

Co-principal investigators in this study were:

Dr. Willard R. Chappell
Director
Environmental Trace Substances Research Program
University of Colorado

Dr. Robert H. Shapiro
Professor
Department of Chemistry
University of Colorado

Dr. Robert E. Sievers
Professor
Department of Chemistry
University of Colorado

Dr. Robert Barkley played a key role in the GC/MS analyses and in the interpretation of the data. Several graduate students were involved. Section 4, "Model Compounds: Xanthine and Pyramide Derivatives", is a shortened version of the Ph.D. thesis of Dr. Kenneth Kolonko.

SECTION 2

CONCLUSIONS

Studies of the influent to the ozonation basin at the UTSD facility have shown the presence of halogenated hydrocarbons, sulfides, aromatic hydrocarbons, alkanes, alkenes, fatty acids, fatty acid esters, phthalate esters, phenols and several other individual compounds. Treatment with ozone did not cause any compounds that had been identified in the influent to be removed completely. However, those compounds produced by ozonation of water at UTSD indicate that at least some constituents were partly destroyed. Those compounds most susceptible to treatment with ozone include unsaturated hydrocarbons and unsaturated fatty acids and esters. It is apparent from these studies that insufficient ozone was used at the UTSD treatment facility to meet total chemical oxygen demand and thus, only those compounds most easily oxidized by ozone were reduced in concentration.

Several classes of organic compounds are formed in UTSD wastewater after treatment with ozone. Samples of water treated in the UTSD facility show the presence of aldehydes, fatty acids, alkanes and aromatic hydrocarbons at concentrations much greater than before treatment. Compounds largely unchanged by ozonation under UTSD plant conditions include aromatic hydrocarbons, chlorinated hydrocarbons, alkanes and fatty acids.

Although ozone treatment at the UTSD plant apparently is adequate to disinfect the effluent, sufficient excess ozone is not present to meet total chemical ozone demand. Laboratory studies, however, indicate that if ozone contact time and doses were increased, substantially greater changes would occur in the chemical composition of UTSD wastewater. In the limit these changes would include destruction of unsaturated compounds, xanthines (such as caffeine), aldehydes, and alcohols. Greater amounts of many compounds would be formed, including aldehydes, alkanes, acids, and aromatic hydrocarbons. No determination has been made as to the necessity or desirability of treating with more ozone than is necessary to effect disinfection.

Additional compounds have been identified in other unozonated water systems, including paper mill wastewaters, domestic potable waters and effluents from a speciality chemical company and a large chemical manufacturing site. After treatment with ozone in the laboratory the following classes of

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compounds were determined to be destroyed: sulfides, sulfoxides and phosphonate esters.

Laboratory studies of model compounds have shown that caffeine and other xanthines are attacked by ozone to yield triazine and imidazoline derivatives. Unsaturated hydrocarbons and derivatives yield aldehydes, alkanes, and acids. Sulfides and sulfoxides produce sulfones when treated with ozone, yet phosphonate esters are only partially oxidized. The above are all relatively stable oxidation products of ozone treatment under conditions expected in a wastewater treatment facility.

Evidence for relatively long-lived free radical species has been obtained from samples of ozonated wastewater under both UTSD and laboratory reaction conditions. Mechanistic studies of the ozonation of xanthine derivatives, show intermediate N-formylamines (amides) are produced, but are further oxidized upon prolonged ozone treatment. Aldehydes, produced from unsaturated compounds, also are intermediates which are further decomposed by ozone to yield alkanes and acids. Other possible intermediate species including ozonides, peroxides and hydroperoxides were not identified during this study. Since these long-lived intermediate species are of potential importance in determining the environmental consequences of using ozone to disinfect water, additional study is recommended to determine the lifetimes of such species downstream from the treatment plant.

High concentrations of added chloride ion were not found to cause production of chlorine containing products in ozonated wastewater.

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SECTION 3

WASTEWATER ANALYSIS

The primary scientific objective of the work reported herein was to determine the chemical changes which occur when wastewater is subjected to ozone treatment. A major effort was made to identify organic chemical components of wastewater both before and after treatment with ozone. The information obtained from these studies is useful not only to delineate wastewater composition, but also to suggest possible reactant/product relationships that are indicative of the chemistry which occurs in the disinfection process.

For pragmatic purposes, the organic compounds obtained from water samples generally are considered in two classes: volatile and non-volatile. Separation between these two classes is not always clear; some overlap exists and, in practice, this is desirable because various analytical methods are more or less efficient for a given compound. The state of the art is such that volatile compounds, i.e., those purgeable by the method described below, are easier to concentrate and identify than non-volatile organics. The emphasis in this work was to determine the changes that occur in the volatile constituents of the samples obtained. In addition, the non-volatile components in some samples were concentrated by several methods (see below) and many non-volatile compounds that are amenable to gas chromatographic separation were identified. Unlike analyses for volatile constituents, however, non-volatiles were not determined on a routine basis because the methodology is time consuming and not highly sensitive for sub-part per billion concentrations.

Gas chromatography was used to separate the complex mixtures which were concentrated from the various water samples. Generally, chromatographic conditions and retention times were determined using a chromatograph equipped with a flame ionization detector. In most cases, these measurements were followed by combined gas chromatography/mass spectrometry (GC/MS) analysis in order to confirm the identity of compounds.

Ozonated and unozonated wastewater from the Upper Thompson Sanitation District (UTSD) treatment plant at Estes Park, Colorado, provided the majority of samples studied in this work. Additional samples for laboratory ozonation studies were obtained, as available, from several sources. One

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objective of the latter work was to determine the chemical changes caused by ozonation of various industrial wastewaters. Such samples were obtained from a paper mill source, a sugar mill source, the effluents of a large chemical concern in the Denver, Colorado, area, and a small Boulder, Colorado, specialty chemical manufacturer. A domestic wastewater sample, heavily contaminated with paper mill effluent was also obtained and used in this work.

A related experiment entailed the determination of ozone oxidizing power lifetimes in aqueous solutions. Another was a study of the effect of the combination of ozone and ultraviolet radiation on wastewater chemistry. These experiments were performed with model compounds treated under laboratory conditions. Exhaustive studies of additional model compounds were made using ozone alone.

ANALYTICAL METHODS

Concentration of Volatile Organics

Volatile organic components were purged from water samples using a device patterned after that used at the National Bureau of Standards.[18] The device, including our modifications, is illustrated in Figure 1. The apparatus consists of a 2-liter, 3-neck round bottom flask fitted with a thermometer, a fritted gas dispersion tube, and a borosilicate glass tube filled with a porous polymer sorbent. A heating mantle with a variable power supply provided heat to the flask. A manifold was used to join four apparatus together so that four samples could be concentrated simultaneously (Figure 2). The purge gas, generally nitrogen, was first passed through a trap of molecular sieve 5A and then through a trap of Tenax-GC, the same porous polymer used in the sorbent traps. The latter trap was used to remove potential impurities in the purge gas that otherwise would be concentrated in the analytical trap.

Sorbent traps were fabricated from fire polished borosilicate glass tubes 17.5 cm x 4 mm ID x 1/4 in. OD. Glass wool plugs were used to retain the porous polymer in the tube (Figure 3). Compression fittings with PTFE ferrules were used to seal the tubes and to make all connections in the purging apparatus. The porous polymer (poly-2,6-diphenyl-*p*-phenylene oxide) Tenax-GC, was used as the sorbent material. Generally, sorbent traps used in this work contained 150 mg of Tenax-GC. Before use, each trap was conditioned initially at 200°C with nitrogen gas flow at 100 mL min^{-1}.

Tenax-GC was described first in 1970 by VanWijk.[19] It was chosen for this work because it has been used widely[20] to sample trace organics from aqueous media. Tenax exhibits high thermal stability and low bleed, is inert to oxygen, and has little affinity for water. The polymer has a specific

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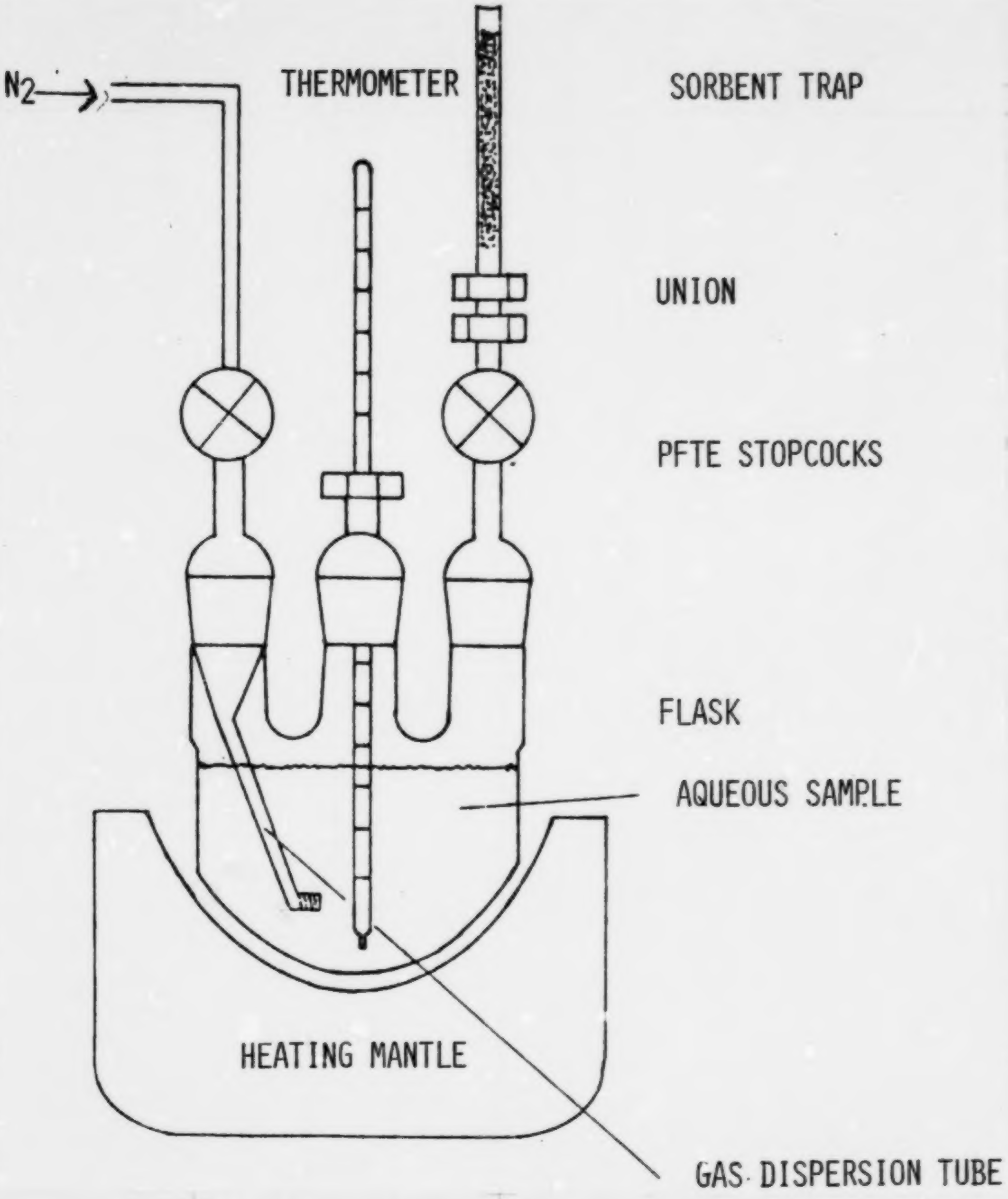
N_2
THERMOMETER
SORBENT TRAP
UNION
PFTE STOPCOCKS
FLASK
AQUEOUS SAMPLE
HEATING MANTLE
GAS DISPERSION TUBE

Figure 1. Purge device and sorbent trap for concentrating trace level volatile organic material in aqueous solution.

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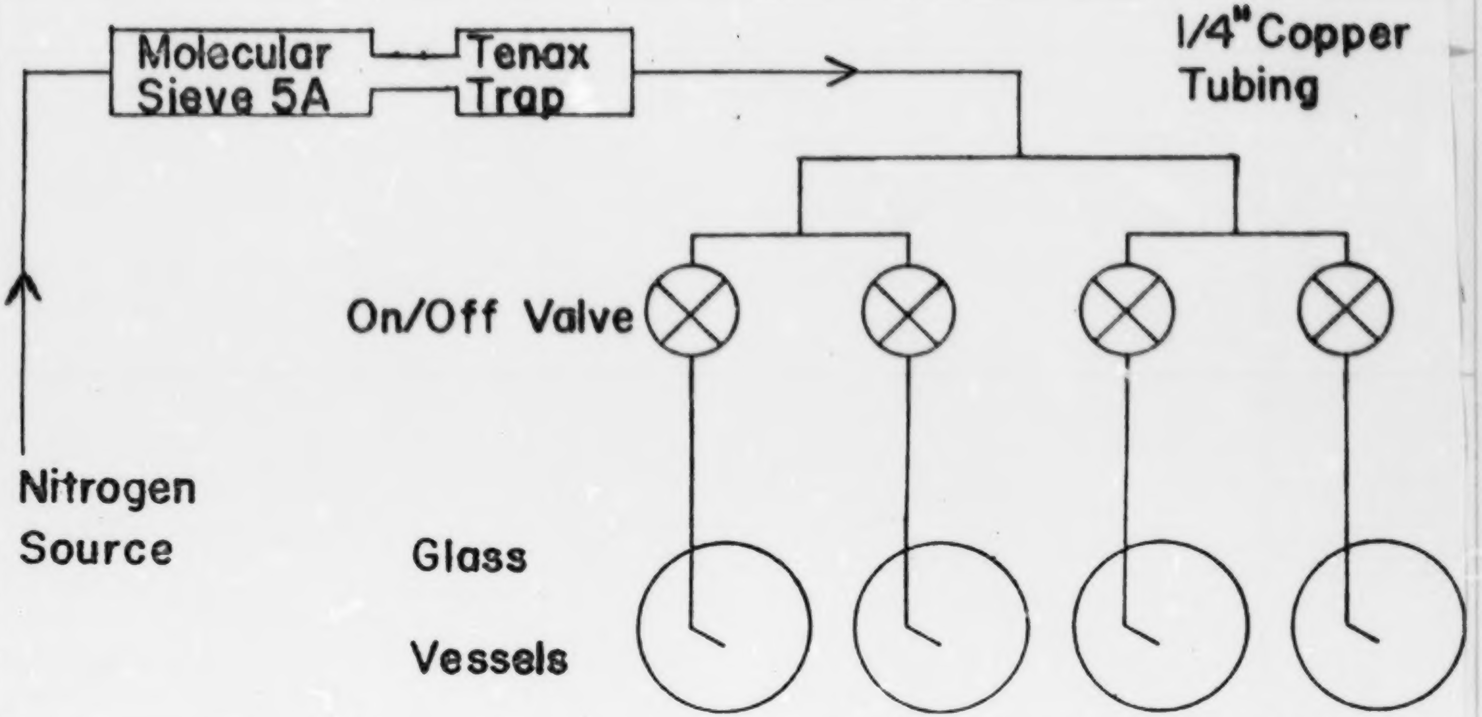
Molecular Sieve 5A
Tenax Trap
1/4" Copper Tubing
On/Off Valve
Nitrogen Source
Glass Vessels

Figure 2. Manifold of purge and trap apparatus.

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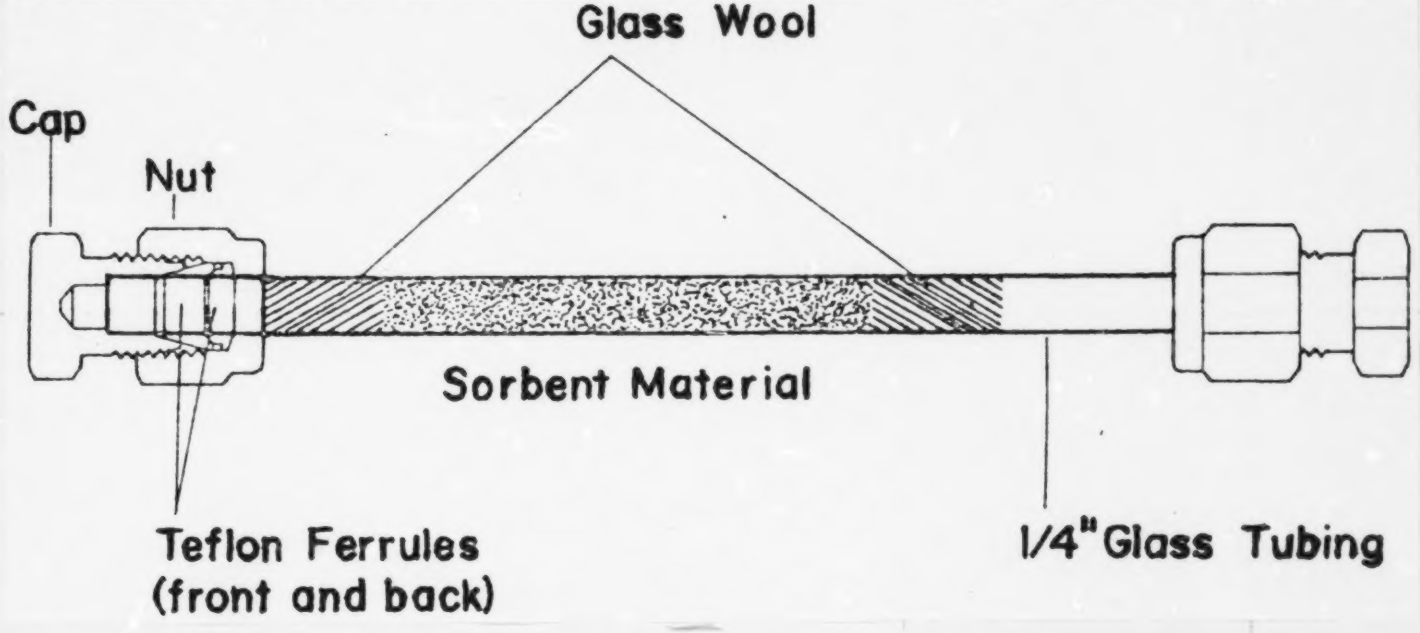
Glass Wool
Cap
Nut
Sorbent Material
Teflon Ferrules
(front and back)
1/4" Glass Tubing

Figure 3. Sorbent trap used to concentrate volatile organic material.

surface area of 18.6 m^2 g^{-1}, an average pore size of 720 Å, a pore volume of 0.67 cm^3 g^{-1}, and a molecular weight range of $0.5 - 1 \times 10^6$ g mol^{-1}.

A typical sample was collected and concentrated by the following procedure. Samples were collected in 1-gallon amber glass bottles that had been washed three times with a two percent aqueous solution of Alconox® laboratory detergent. The detergent was rinsed from the bottles three times with hot tap water and then three times with deionized and distilled water. Washed sample bottles were sealed with aluminum foil and screw caps. A sample was taken by first rinsing with two aliquots of sample water and then filling the bottle to overflowing. Filled bottles were sealed with aluminum foil and screw caps. Samples were transported to the laboratory in ice and stored in a refrigerator at 4 °C. Analyses usually were made within 24 hours.

A purging apparatus, which had been cleaned using the same procedure as for the sample bottles and oven dried, was rinsed with two small aliquots of sample. The flask was next filled with a portion of the sample, generally one liter. A sorbent trap was attached and the purge gas flow was adjusted to 250 mL min^{-1}. The heating mantle was turned on and the samples purged for 20 minutes at which time the water temperature had reached 70 °C. The traps were removed from the flask and water removed by drying at room temperature for five minutes with a nitrogen gas flow of 50-100 mL min^{-1}. These traps were capped and stored in a refrigerator at 4 °C until gas chromatographic separation was performed.

The purging procedure described above relates the optimum conditions that we have determined for survey analyses of the compounds we found most commonly in wastewater samples. The conditions are not necessarily the optimum ones to use for a specific component, but do yield reliable data for a range of compounds including low molecular weight halocarbons, aromatic hydrocarbons, saturated hydrocarbons and some oxygenated hydrocarbons. It should be noted that this analytical procedure could cause the destruction of any reactive intermediate species resulting from treatment with ozone. However, hydrolytic decomposition of ozonation intermediates[12] which could occur as a result of the analytical procedure would also occur in treated wastewater effluent. The compounds determined in this analytical procedure are the stable end-products of ozonation. For one-liter samples, we could determine less than 0.1 μg of most volatile components in routine testing.

Concentration of Non-volatile Organics

Several methods were tested for concentrating non-volatile trace level organic components of wastewater.

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Liquid-Liquid Extraction--

One preliminary experiment was performed using a conventional solvent extraction technique with chloroform. Although the solvent used was a highly pure, distilled in glass, chemical, the solvent blank gave backgrounds which were unacceptable for routine analyses of this type. The data which were obtained in this experiment, however, were useful to establish a compound suitable for a laboratory scale model compound study.

Steam Distillation--

Two different steam distillation-extraction devices were constructed and used in this work. One apparatus, using a solvent lighter than water, was based on the work of Veith and Kiwus.[21] We designed and constructed a similar device which employs a solvent heavier than water (see Figure 4). Both devices work in the same manner. Water containing organic components is boiled and the resulting steam carries over the organics which condense and are extracted by the organic solvent. Water is recycled to the distillation flask to complete the continuous cycle. The apparatus is designed to concentrate the organic components from a large sample into a volume of organic solvent extraction. Generally we used only 10 to 20 mL of an appropriate organic solvent. This small amount of solvent, when concentrated to less than 1 mL, gives a chromatographic baseline virtually free of peaks. In addition, many components of water samples that are easily removed in a solvent extraction and interfere with chromatographic analyses are not concentrated by the steam distillation procedure.

Concentration on Porous Polymers--

Several porous polymers are useful to concentrate trace level organics from aqueous solution. The technique generally requires that solution be passed through a bed of resin and the concentrate eluted with an organic solvent. The solvent containing the sample components is evaporated to a small volume and the sample injected on a gas chromatograph to separate the components. We have used the XAD series of resins: XAD 2, 4, and 8, and a combination of 4 and 8 followed by methanol or ether extraction.

Methylation Techniques--

Many of the compounds that were obtained by the methods described in this section are not amenable to gas chromatographic separation unless more volatile derivatives are made. During the course of this work we used two different methylation techniques. One technique using a diazomethane was useful to methylate extracted compounds in non-aqueous solvents. Another technique for performing methylation in aqueous solvents used dimethylsulfate. The diazomethane procedure is well known from the literature.

The dimethylsulfate procedure, however, has not been used extensively, although Keith made an exhaustive study of effluents using this method followed by solvent extraction.[22] We used a different methylation procedure that

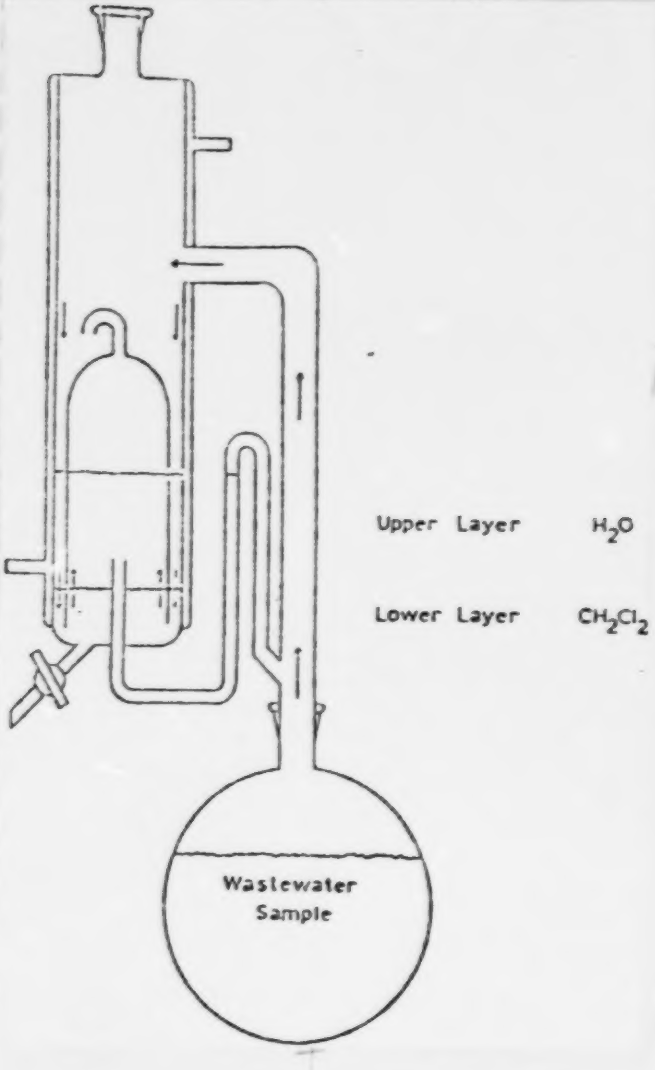
Upper Layer H_2O
Lower Layer CH_2Cl_2
Wastewater Sample

Figure 4. Steam distillation apparatus for use with solvents having a density greater than water.

was adapted from Keith and has not been reported previously. The pH of the water sample was raised to 11 with 50% sodium hydroxide. The sample was heated to 85°C and 40 mL of freshly distilled dimethylsulfate was added while maintaining the pH in the range 10 to 11. After one hour, unreacted dimethylsulfate was destroyed by adding 5 mL of concentrated ammonium hydroxide. (CAUTION: Dimethylsulfate is carcinogenic. Inhaling this strong alkylating agent has been linked to pulmonary carcinomas.) After methylation, we purged the sample as described in the previous section and sorbed the volatiles on a trap of Tenax-GC. The method is particularly advantageous when sampling water sources with substantial amounts of phenolic and carboxylic species.

Analytical Instrumentation

Open Tubular Chromatography Columns--

Glass capillary chromatographic columns were prepared from tubular borosilicate stock using a Hewlett-Packard Hupe-Busch 1045A Capillary Drawing Machine. We found that columns with approximately 1/16 in. OD were less susceptible to breakage. The internal diameter was 0.25 mm and the columns were drawn to approximately 30 m. Before coating, the interior of the capillary was deactivated by treatment with 1% benzyltriphenylphosphonium chloride. This solution was removed by flushing with water and the column was baked at 250 °C for two hours with nitrogen flow. One-fourth of the length of the column was filled with a 10% solution of OV-101 in chloroform. The plug of liquid solution was forced through the column at a rate of 20 mm sec^{-1}. Nitrogen flow was maintained for twelve hours at ambient temperature. Finally, the temperature was increased at 1 °C min^{-1} to 250 °C and held for one hour.

Instrument Modification--

A Valco Company, 10-port high-temperature gas switching valve was installed in a Varian Model 2400 gas chromatograph which was modified further to accept a capillary column. Appropriate detector and injection port liners were installed to reduce dead volume, and a make-up gas tee was added at the flame ionization detector. An aluminum block heating oven was constructed in such a way that it could be used to rapidly heat the sorbent tubes when they were attached to the gas switching valve.

Similar modifications were made to the chromatograph of a Hewlett-Packard 5982A GC/MS/computer system. The GC/MS interface was modified by installation of 0.5 mm ID x 1/16 in. OD glass lined stainless steel transfer lines between the capillary column and the mass spectrometer. Micro needle valves were installed to allow the column to vent to the atmosphere when not in use. The capillary column was linked directly to the ion source and no carrier gas separator was used.

Various other gas chromatographs were used as necessary to complete some experiments. These instruments were equipped with either packed columns or capillary columns as appropriate to each experiment.

Chromatographic Procedures

The analytical sample was back-flushed from the sorbent trap to the chromatographic column by a thermal desorption procedure. The sorbent trap was connected to the gas switching valve and a desorption oven was placed around the trap. The oven was maintained at 180 °C. The valve was changed so that carrier gas was diverted through the sorbent trap and then to the chromatographic column. During this procedure, the chromatographic oven was maintained at -40 °C (as monitored by a thermocouple) using solid carbon dioxide as the coolant. Coolant was placed directly in the chromatograph oven, but contact with the column was avoided. The chromatograph of the GC/MS was cooled with a liquid carbon dioxide sub-ambient cooling system. At -40°C the desorbed sample was concentrated at the head of the chromatographic column. The desorption procedure required a minimum of five minutes, at which time the switching valve was changed to remove the sorbent trap from the gas flow path. These and other operating parameters for the Varian 2400 are given in Table 1.

The GC/MS system was operated in substantially the same manner as the FID chromatograph. The data system was programmed to operate the mass spectrometer with the following conditions.

Mass Range: 35 or 45-300 mass units
Scan Time: 2-4 S
A/D Measurement: 2 or 3
Mass Offset: determined daily
Peak Detection Thresholds: 9,9

Data were stored in the computer system. Compound identifications were based on manual and computer comparison with reference spectra. Identifications were confirmed by comparison of retention data and spectra of known compounds determined in our laboratory.

Quantitative Procedures

Standards for quantitative analysis were prepared in methanol solution and added to purified water. The standard compounds were purged from aqueous solution by the same procedure used for an actual sample. Methanol is not retained by Tenax-GC and did not interfere. Purified water was prepared by passing laboratory deionized and distilled water through a bed of XAD-2 resin and redistilling in an all-glass still. The resin was regenerated periodically as indicated by analysis of a water blank. Stock solution of a series of

TABLE 1. GAS CHROMATOGRAPH INSTRUMENT PARAMETERS

Function	Stand-By	Operating	
		Load	By-Pass
Power	ON	ON	ON
Injector Temperature	180 °C	180 °C	180 °C
Detector Temperature	180 °C	180 °C	180 °C
Gas Switching Valve (GSV) Temperature	180 °C	180 °C	180 °C
Desorption Oven Temperature	185 °C	185 °C	185 °C
Linear Temperature Programmer	Iso	Off	Hold
Column Oven Temperature	100 °C	-40 °C	0 °C
Program Rate (°C min^{-1})	1	1	1
Inlet Line Temperature	25 °C	100 °C	100 °C
GSV Mode	By-Pass	Load	By-Pass
Column Inlet Pressure	9 psig*	9 psig*	9 psig*
Split Ratio	4:1	4:1	4:1
Hydrogen Flow (mL min^{-1})	0	30	30
Air Flow (mL min^{-1})	0	300	300
Make-up Flow (mL min^{-1})	0	30	30
Chart Rate (in. hr^{-1})	Off	8	8
Cell Voltage	F1	F1	F1

* psig is pounds per square inch gauge

standards by volumetric dilution is given in Table 2. For quantitative data obtained with this procedure, it is assumed that the same purging efficiencies exist for actual samples and those prepared in the laboratory. We have made a more thorough study of the quantitative aspects of this method as a part of another study conducted in our laboratory. These data are given in another report by Walton[23] and a thesis by Eiceman.[24]

TABLE 2. VOLATILE ORGANICS IN STOCK SOLUTION. LISTED BY ELUTION ORDER ON OV-101 COLUMN

Compound	Density$^{20}_{4}$	M.W.	Volume µL	Concentration ng/µL
n-Pentane	0.6262	72.15	5.0	310
Chloroform	1.483	119.4	10.0	1500
1,2-Dichloroethane	1.235	98.96	10.0	1200
Benzene	0.8786	78.12	2.0	180
Trichloroethylene	1.464	131.4	10.0	1500
n-Heptane	0.6837	100.2	5.0	340
Bromotrichloromethane	2.012	198.3	10.0	2000
Toluene	0.8669	92.15	2.0	170
Tetrachloroethylene	1.623	165.8	10.0	1600
n-Octane	0.7025	114.2	15M*	210
1-Bromopentane	1.218	151.1	5.0	600
Chlorobenzane	1.106	112.6	5.0	550
p-Xylene	0.8611	106.2	5.0	430
Ethylbenzene	0.8670	106.2	5.0	430
o-Xylene	0.8802	106.2	5.0	440
n-Nonane	0.7176	128.3	15M*	210
1-Bromohexane	1.174	165.1	5.0	590
p-Chlorotoluene	1.070	126.6	5.0	540
m-Dichlorobenzene	1.288	147.0	5.0	640
n-Decane	0.7300	142.3	15M*	210
n-Undecane	0.7402	156.3	15M*	210
n-Dodecane	0.7478	170.3	15M*	210

15M* is 15 microliters of a 20 percent by weight mixture of n-octane, n-nonane, n-decane, n-undecane, and n-dodecane. Fifteen microliters has a mass of 0.0105 grams. Source for physical data is - Atlas of Spectral Data and Physical Constants for Organic Compounds, J.G. Grasselli, CRC Press, 1973.

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OZONATION OF WASTEWATER

Upper Thompson Sanitation District

The wastewater treatment plant operated at Estes Park by the UTSD is designed to accommodate 1.5 million gallons per day (mgd). The facility is similar to other wastewater treatment facilities except that ozone, rather than chlorine, is used as the disinfection reagent prior to discharge of effluent from the plant.

The major components of the treatment process are: flow equalization, activated sludge treatment, biological nitrification, multi-media filtration and disinfection by ozone. These components have been studied and evaluated separately by the UTSD and M & I Consulting Engineers, Inc. under a separate contract (EPA R-803831-01) entitled "An Evaluation of Pollution Control Processes - Upper Thompson Sanitation District".

Before discharge to the Big Thompson River, the effluent is contacted with ozone in a covered and ventilated concrete tank 38 feet long by 4.5 feet wide x 12 feet deep. Baffles divided the tank into 9 compartments such that an over-under flow of water is achieved. Ozone is admitted to each compartment using four ozone diffuser tubes. At the design capacity (1.5 mgd) this 15,350 gallon tank provides a detention time of 15 min.

In the comparative studies described in this report, samples were obtained prior to the inlet to the ozonation basin and at the last sample tap in the ozonation basin.

The UTSD treatment facility provides services to the area surrounding Estes Park, Colorado, near Rocky Mountain National Park. Except for small business, including service stations and other service facilities which may use small amounts of chemicals, no industrial effluent is known to the treatment facility. The domestic waste effluent to the facility includes septage pumping and previously chlorinated wastes from the National Park Service. In addition, previously chlorinated wastes from the Estes Park Sanitation District were used routinely to meet the minimum flow (0.75 mgd) required for evaluation by M and I, Inc.

As with any new facility, various start-up problems were expected with this treatment plant. Although several problems led to a projection that the plant would function properly by January, 1977, in fact continuing problems associated with ozone production at the plant created severe problems in this study because samples could be obtained only on an irregular basis. Therefore, it was very difficult to complete as thorough a study as originally intended. Details of the problems associated with operation of the plant are to be presented in the report to be made under EPA contract R-803831-01.

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Wastewater samples were obtained from the UTSD treatment facility as available to obtain a profile of the changes which occur when domestic wastewater is subjected to treatment with ozone. Typical chromatograms of the volatile components are shown in Figures 5 and 6. The data are presented as mass-resolved chromatograms after the method of Biller and Biemann.[25] Chromatograms from different samples indicate that the chemistry which occurs under ozonation plant conditions is similar for a variety of compounds on a long term basis. The numbered compounds in the chromatograms are listed in Table 3.

TABLE 3. VOLATILE COMPOUNDS IDENTIFIED IN UTSD WATER SAMPLES

Peak	Compound	Approximate Conc./ppb Before Ozonation (Fig. 5)	Approximate Conc./ppb After Ozonation (Fig. 6)
1	Chloroform	0.2	0.03
2	1,2-Dichloroethane	b	b
3	Carbon tetrachloride	b	a
4	n-Hexane	b	a
5	Bromodichloromethane	b	b
6	Trichloroethylene	b	b
7	Dimethyl disulfide	b	b
8	Toluene	0.5	0.1
9	Heptane	a	b
10	Tetrachloroethylene	1	0.5
11	n-Octane	a	b
12	n-Hexanal	a	b
13	p-Xylene	0.01	0.01
14	m-Xylene	a	0.02
15	Styrene	0.01	a
16	o-Xylene	0.01	0.01
17	n-Heptanal	a	2
18	Dichlorobenzene isomer	0.2	0.02
19	n-Octanal	a	b
20	n-Nonanal	a	5

(a) This compound not found in this sample.

(b) Compound identified in chromatogram but concentration not determined.

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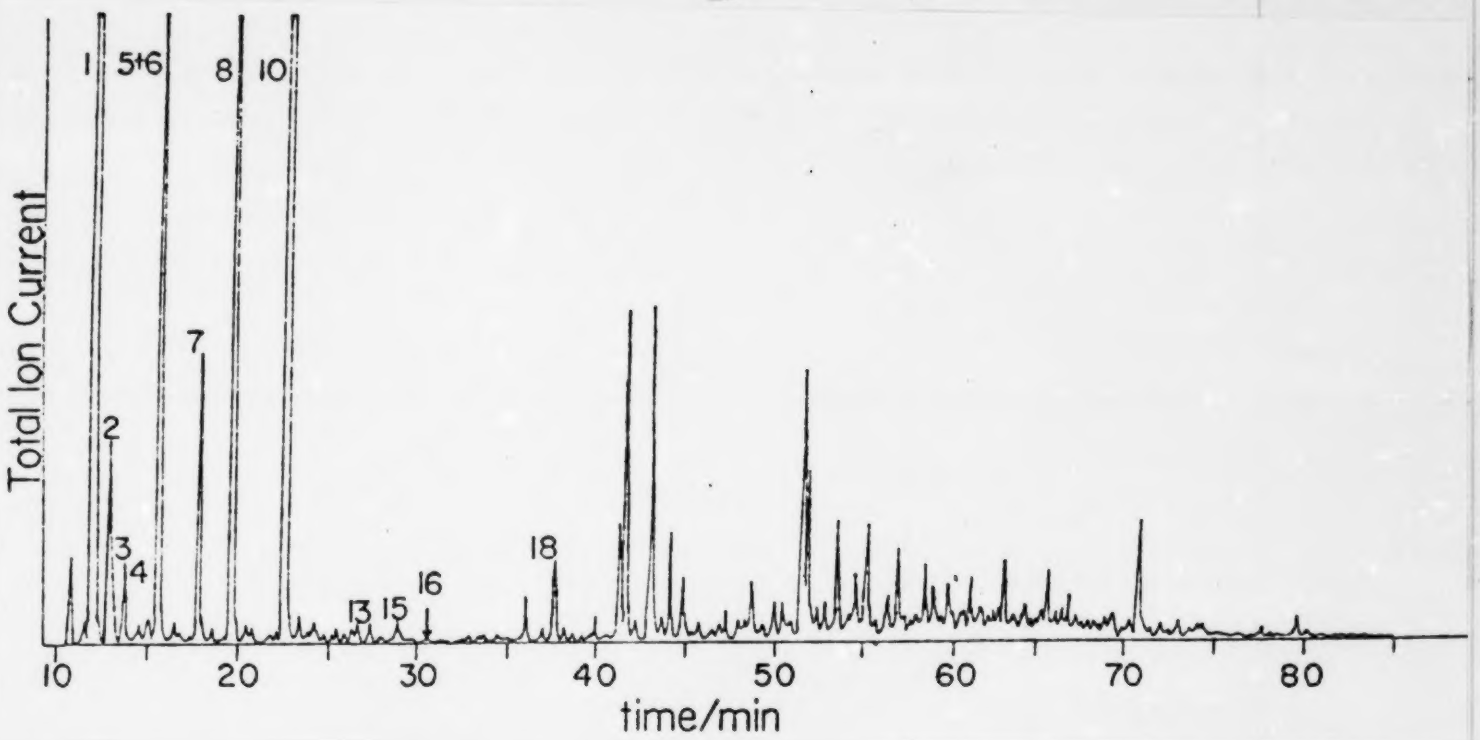

Figure 5. Mass-resolved chromatogram of UTSD wastewater volatiles before ozonation.

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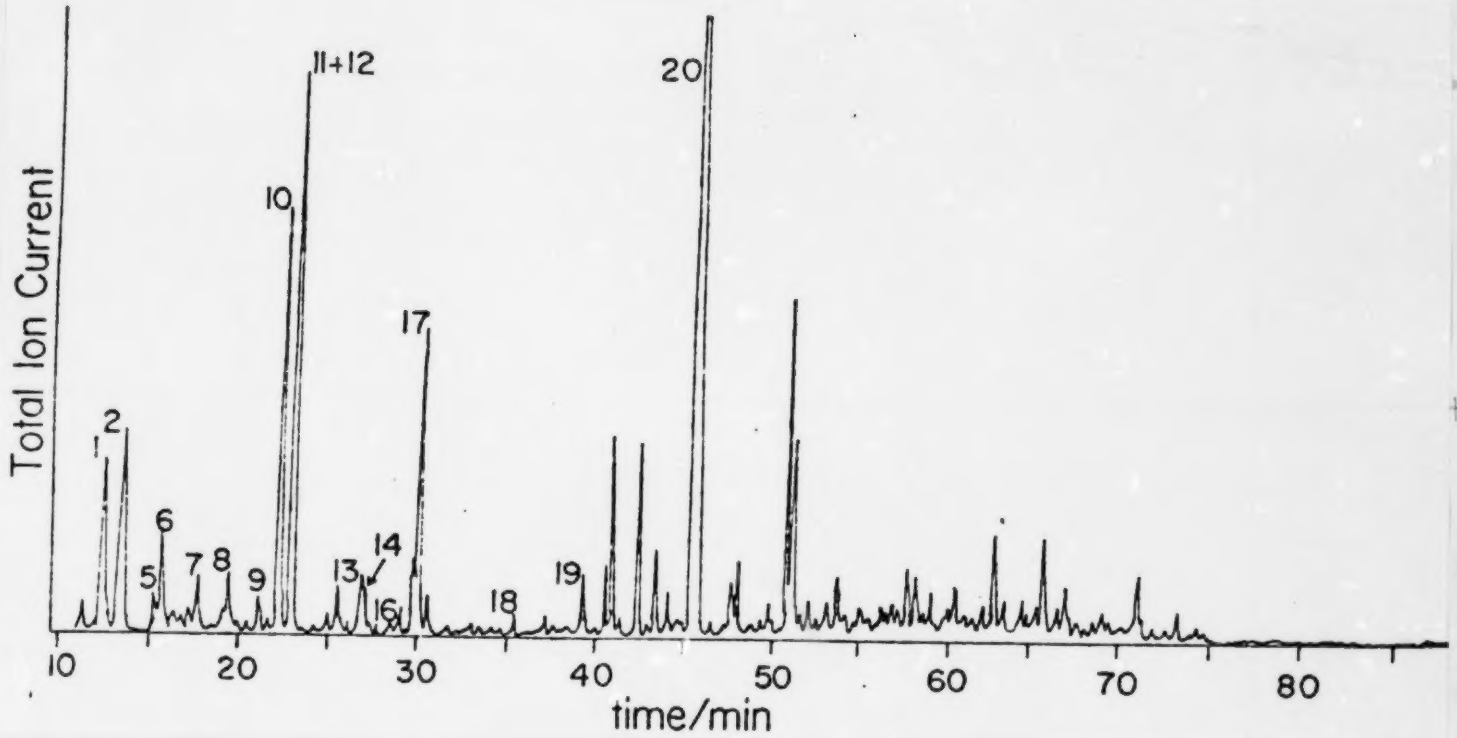

Figure 6. Mass-resolved chromatogram of UTSD wastewater volatiles after ozonation. Numbered peaks given in Table 3.

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The only additional compounds that were identified in other samples of UTSD wastewater are n-pentanal (after ozone treatment) and trimethylbenzene isomers. In general, those compounds which have not been identified in the volatile fractions of UTSD samples give mass spectra indicative of branched hydrocarbons. These components are present both before and after ozonation and do not appear to change upon treatment with ozone.

The additional chromatograms presented in Figures 7 and 8 are data obtained from experiments in which less volatile sample components were concentrated by passing the wastewater through XAD-2 resin and eluting with methanol. The concentrate was methylated before gas chromatographic separation. The compounds are listed in Table 4. Mass chromatograms are also included in these figures to show the different methyl esters found in these samples.

Additional studies were undertaken to measure other parameters associated with ozone treatment of wastewater at UTSD. Some early concern had been expressed that volatile organic compounds produced during treatment may have been purged from the water by the bubbling action during addition of ozone. However, given that the ozone level above the water in the contact basin was found to be in excess of 3 ppm/V (M & I, Inc., measurement) and that laboratory experiments showed that the sampling sorbent (Tenax GC) decomposed upon contact with ozone, no measurements were made of volatile organics above the contact basin.

Several other parameters of interest, apparently all related, also were studied. When samples were taken, the flow of water through the plant was generally much less than the rated capacity. Thus, contact time in the ozonation basin was generally greater than expected for flows equal to the rated capacity. For many of the samples collected here, contact time is estimated to be 0.5 hr. or longer. We suspect that this additional contact time, in part, explains the absence of residual ozone in the contact basin. Both laboratory and on-site measurements of ozone in wastewater from the contact basin showed no detectable ozone. We conclude that the amount of ozone used to treat at UTSD was less or equal to the stoichiometric amount required for all possible ozone chemistry to take place within the contact basin. In contrast, laboratory experiments generally were performed in such a way that residual ozone was present and therefore represent situations where reaction products are not limited by the amount of ozone present.

No attempt was made to determine the relative or absolute amounts of biological or chemical ozone demand. However, our discussion with UTSD and M & I, Inc., personnel indicated that the ozonation system at the treatment facility was adequate for disinfection of the effluent. Overall, it must be concluded that the system, when operating, was functioning properly with respect to biological ozone demand, but that insufficient ozone was present to meet all chemical ozone demand. Our studies at the UTSD plant were intended to determine the chemistry that occurred under ozonation conditions at the plant. Our laboratory studies indicate possible reaction products when sufficient ozone is present in model systems only, not in wastewater systems where there are competitive reactions among large numbers of biological and chemical species.

TABLE 4. COMPOUNDS IDENTIFIED IN METHYLATED XAD-2 CONCENTRATES OF UTSD WASTEWATER

Compound	Retention Time/min Before Ozonation (See Figure 7)	Retention Time/min After Ozonation (See Figure 8)
Nonanoic acid-methyl ester	4.2	*
Decanoic acid-methyl ester	5.4	*
Undecanoic acid-methyl ester	6.4	*
Dodecanoic acid-methyl ester	7.3	*
Tridecanoic acid-methyl ester	8.3	*
Tetradecanoic acid-methyl ester	9.0	9.0+
Pentadecanoic acid-methyl ester	9.7	9.7+
Hexadecanoic acid-methyl ester	10.5	10.5
Hexadecanoic acid-ethyl ester	10.8	10.8
Heptadecanoic acid-methyl ester	11.0	11.0+
Octadecanoic acid-methyl ester	11.7	11.7
Octadecanoic acid-ethyl ester	12.2	12.2
n-Octadecane	9.7	9.7
n-Nonadecane	10.4	10.4
Eicosene isomer	11.0	11.0+
Heneicosene isomer	11.6	*
Docosene isomer	12.4	*
n-Octylphthalate	16.0	16.0

* This compound was not detected in this sample

+ Concentration of this compound was substantially less in this sample than in the sample before ozonation

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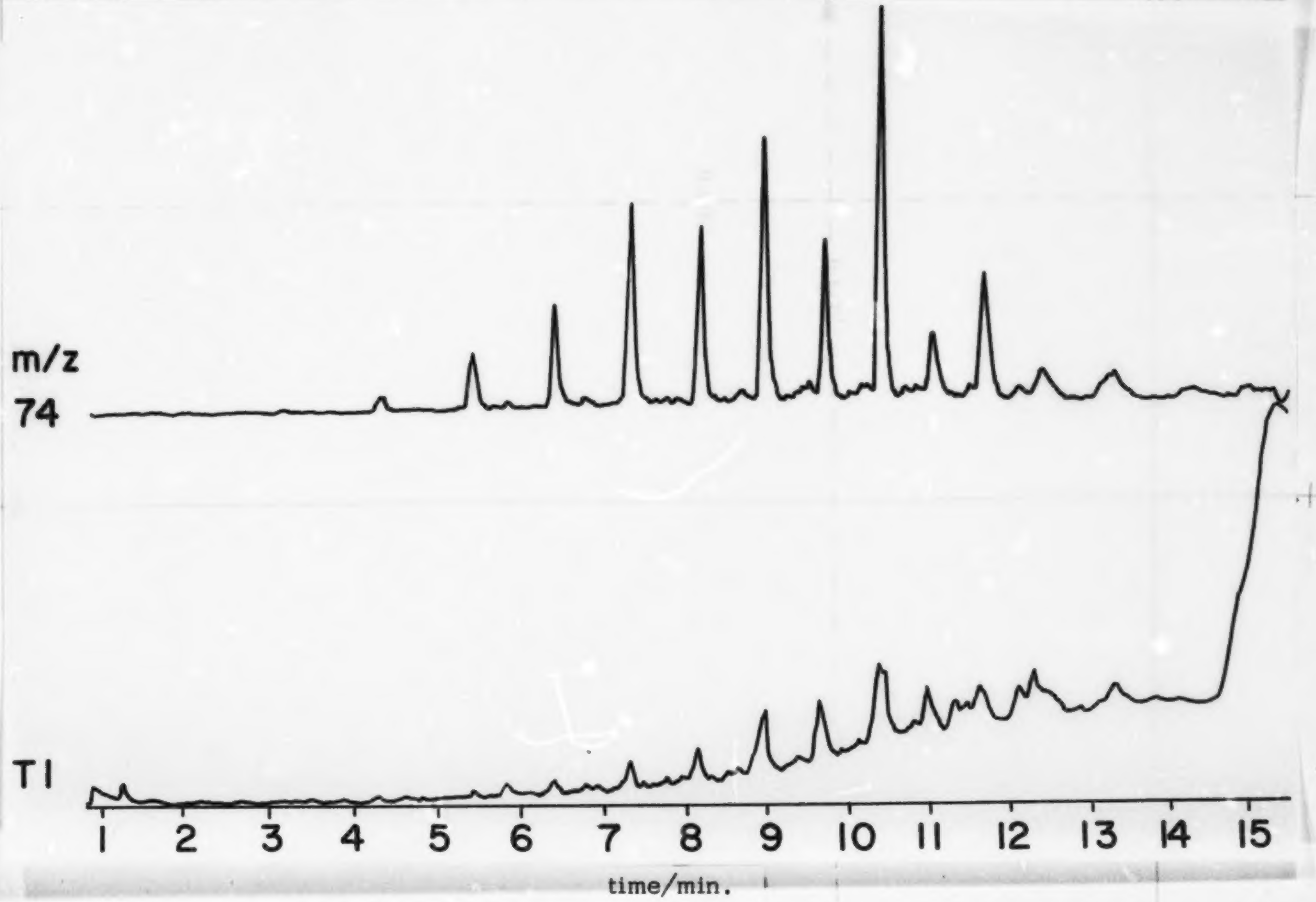

Figure 7. Methylated XAD-2 concentrate of UTSD wastewater before ozonation.

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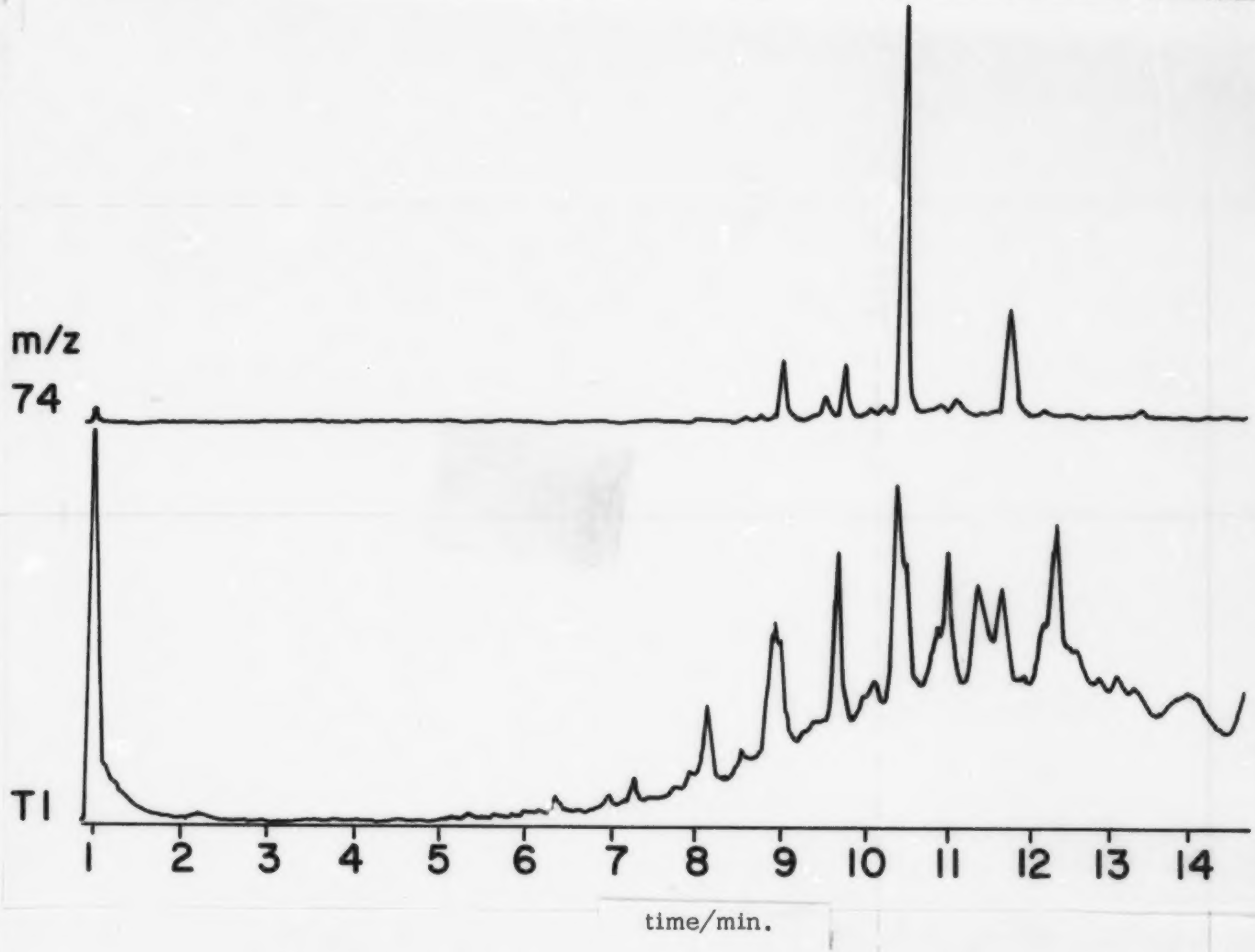

Figure 8. Methylated XAD-2 concentrate of UTSD wastewater after ozonation.

Paper Mill Effluent

A sample of wastewater from a paper mill was provided by EPA-Duluth. Another sample from Port St. Joe, Florida, was provided by the EPA Enforcement Division, Denver, Colorado. The latter sample was a domestic wastewater sample that had a high concentration of paper mill effluent. The paper mill sample was purged and trapped on Tenax-GC both before and after methylation with dimethylsulfate. The sample was treated with ozone and the analyses were repeated. Chromatograms for the unozonated samples are given in Figures 9-11. Identifications for the components are given in Tables 5 and 6. No substantial qualitative changes were observed upon treatment with ozone.

TABLE 5. COMPOUNDS IDENTIFIED IN PAPER MILL WASTEWATER

Peak Number (See Figure 9)	Compound
1	Air
2	Acetone
3	Chloroform
4	Vinyl acetate
5	Pyrrole
6	*n*-Nonane
7	*n*-Propylcyclohexane
8	*n*-Decane
9	*n*-Undecane
10	*n*-Dodecane
11	*n*-Tridecane
12	*n*-Tetradecane
13	β-Caryophyllene
14	*n*-Pentadecane

The Port St. Joe sample was concentrated before and after methylation by the steam distillation technique. Substantial changes were observed when similar analyses were performed after the sample was treated with ozone. Chromatograms are given in Figures 12-15. Representative compounds were identified in these samples and are given in Table 7.

TABLE 6. VOLATILE COMPOUNDS IDENTIFIED IN PAPER MILL WASTEWATER BEFORE AND AFTER METHYLATION

Volatile Compounds (See Figure 10)

Retention Time/Min	Compound
8.0	acetone
10.0	chloroform
12.5	methyl chloroform
14.0	carbon tetrachloride
16.0	methyl isopropylketone
16.5	bromodichloromethane
20.5	dimethyl disulfide
21.0	methyl iso-butyl ketone
23.5	toluene
24.0	pyrrole
24.5	dibromochloromethane
27.0	tetrachlorothylene
32.0	xylene isomer
33.0	xylene isomer
25.5	xylene isomer
37.5	anisole

Volatile Compounds After Methylation (See Figure 11)

Retention Time/Min	Compound Identified as Methyl Derivative (Parent Compound)
8.7	furfuryl-methyl ether (furfural)
12.4	anisole (phenol)
17.3	o-methoxy toluene (o-cresol)
17.9	p-methoxy toluene (p-cresol)
23.4	dimethyl-methoxy benzene (dimethyl phenol)
24.4	o-dimethoxy benzene (catechol)

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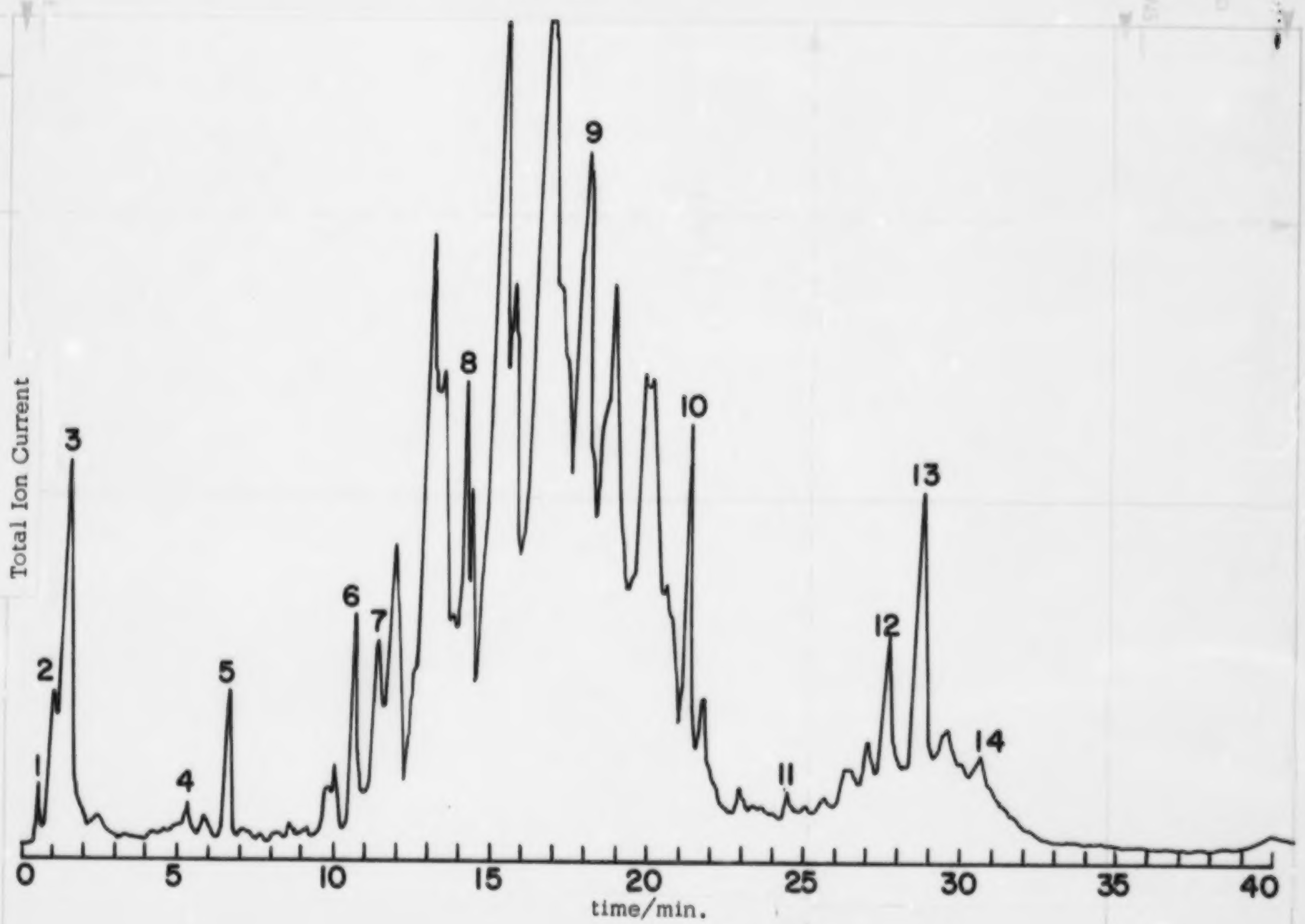

Figure 9. Chromatogram of volatile organic components concentrated from paper mill wastewater.

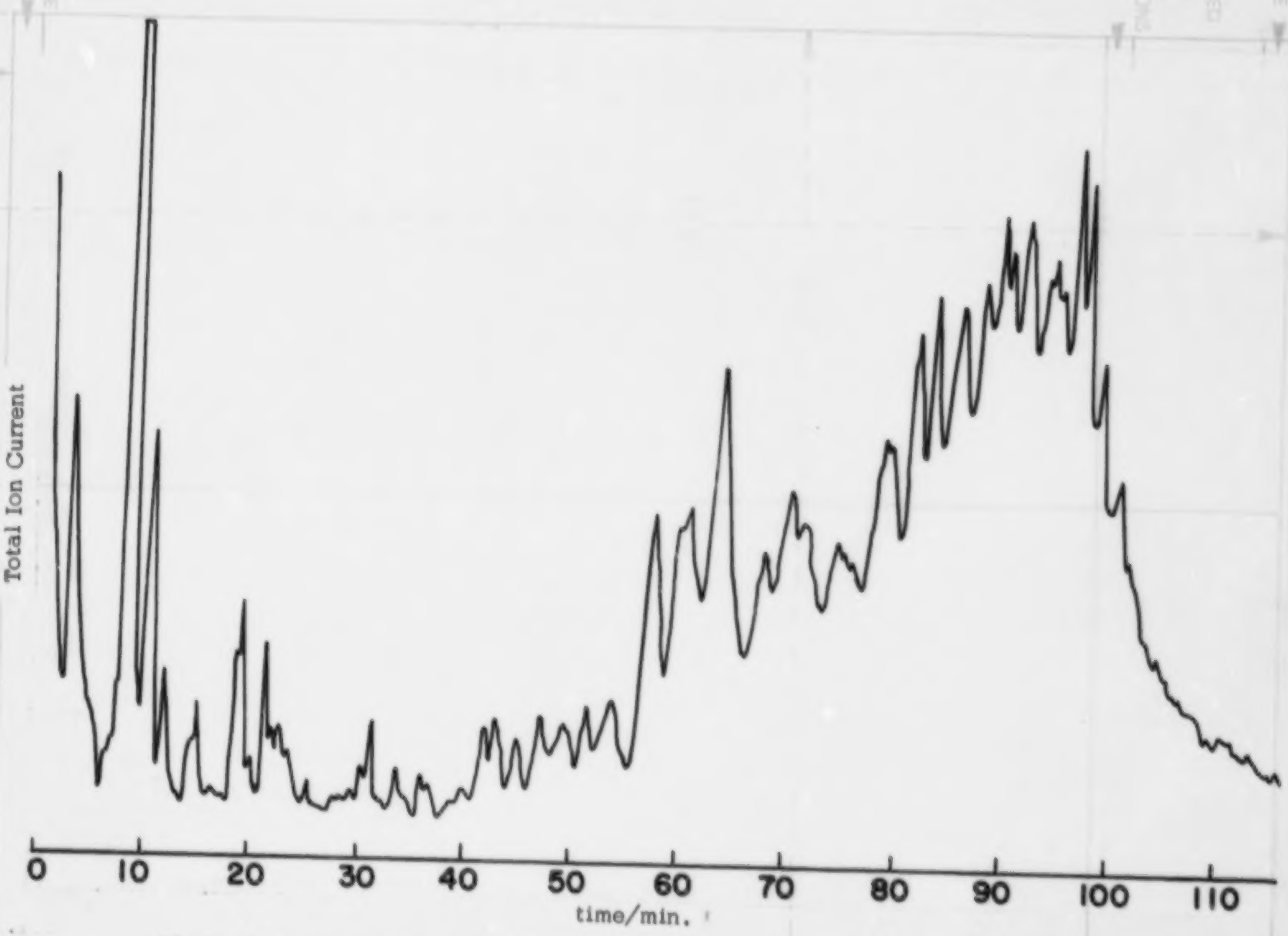

Figure 10. Volatile compounds in paper mill wastewater before methylation.

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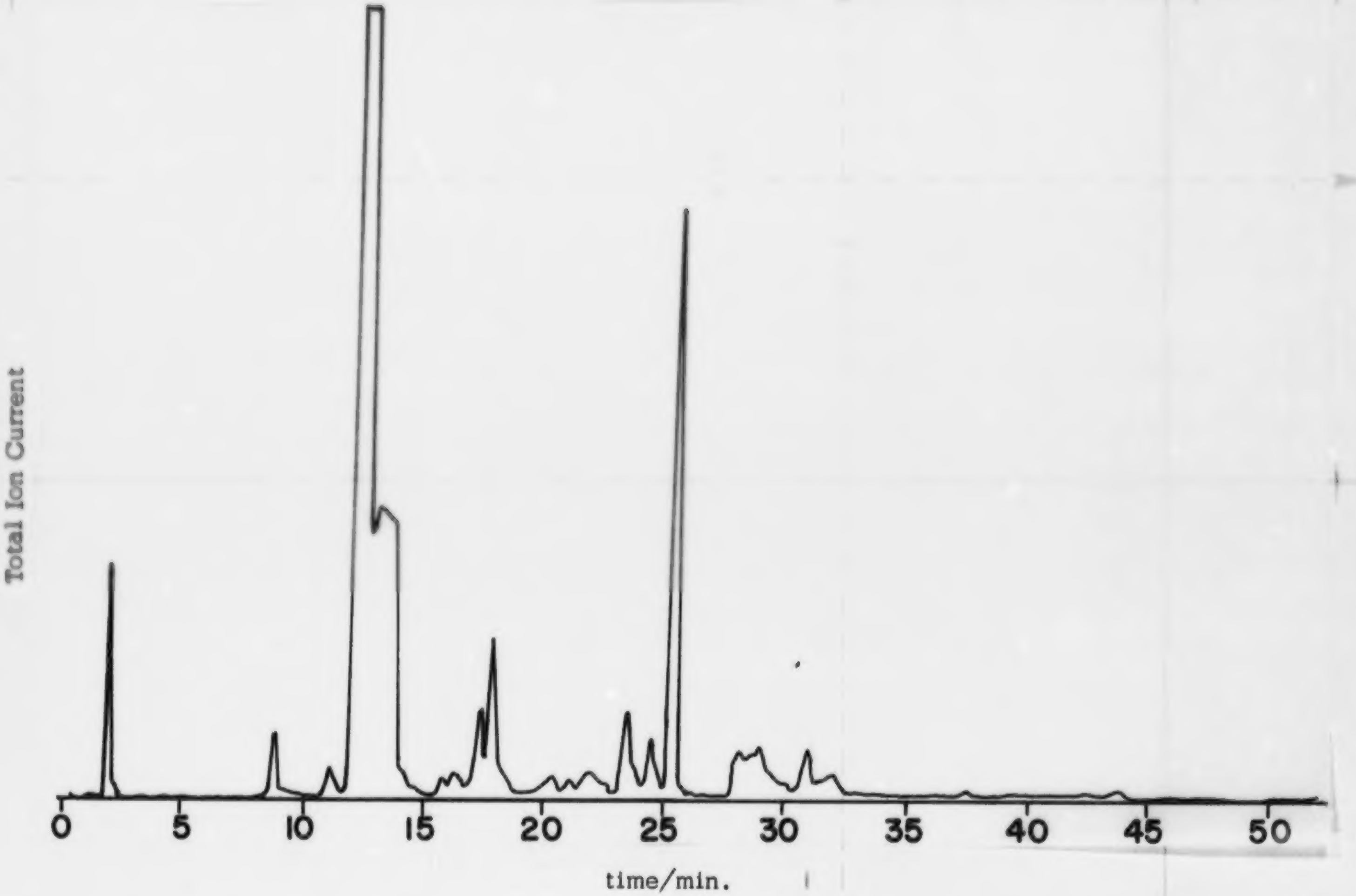

Figure 11. Volatile compounds in paper mill wastewater after methylation.

TABLE 7. COMPOUNDS IDENTIFIED IN PORT ST. JOE, FLORIDA, WASTEWATER (See Figures 12-15).

Peak Designation	Compound
A	Chloroform
B	Dimethyl Disulfide
C	Toluene
D	Tetrachlorethylene
E	Fenchone
F	Camphor
G	β-Carophyllene
H	Dimethyl Trisulfide

Specialty Chemical Company

A sample of wastewater effluent from a specialty chemical company in Boulder, Colorado, was obtained. A dichloromethane extract of this sample was prepared and GC/MS data were obtained as shown in Figure 16. Other aliquots of the sample were treated in the laboratory to determine the effect of ozone and chlorine on this sample. The GC/MS data from these experiments are shown in Figures 17 and 18. The numbered peaks in Figure 16 are listed in Table 8 as well as lettered peaks from Figures 17 and 18. Only those compounds which are formed by either ozone or chlorine treatment are identified in Figures 17 and 18. Other components in these treated samples are substantially identical to those in the untreated sample. The component d,l-2-(6-methoxy-2-naphthyl) propionic acid (peak 23) also is present in treated samples at high concentration and was not allowed to enter the mass spectrometer in order to prevent contamination of the instrument.

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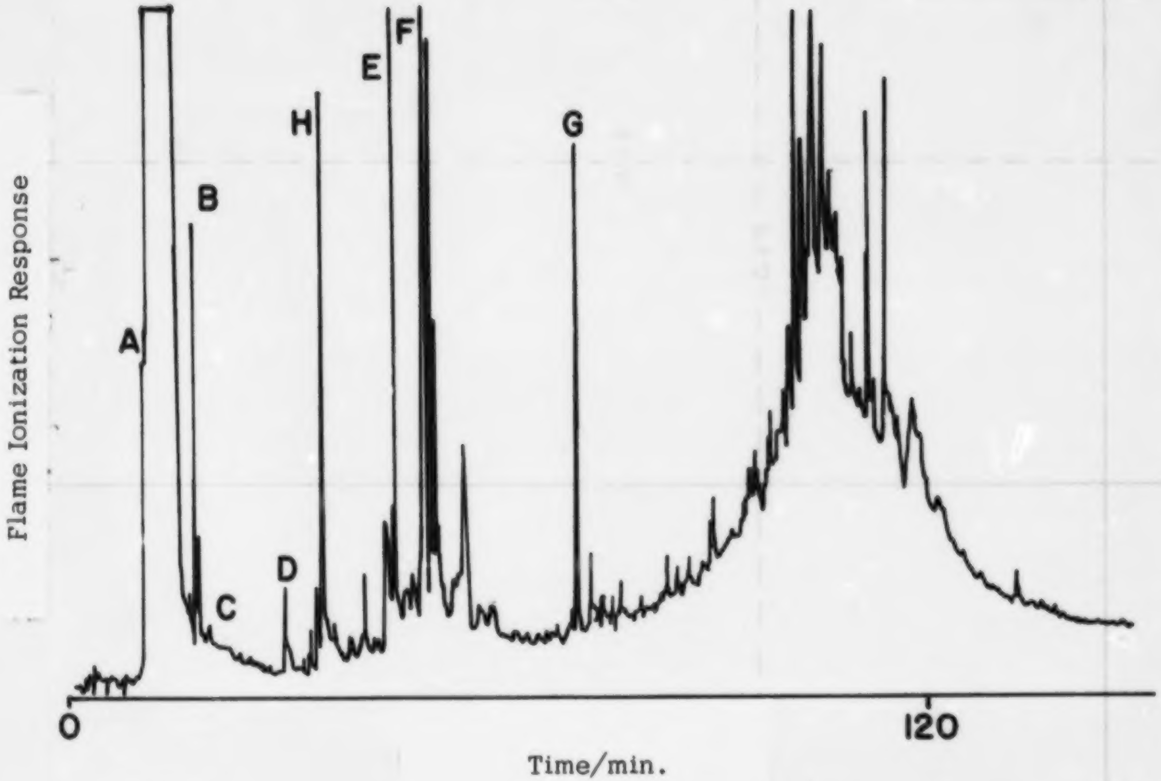

Figure 12. Chromatogram of Port St. Joe, Florida, wastewater concentrate.

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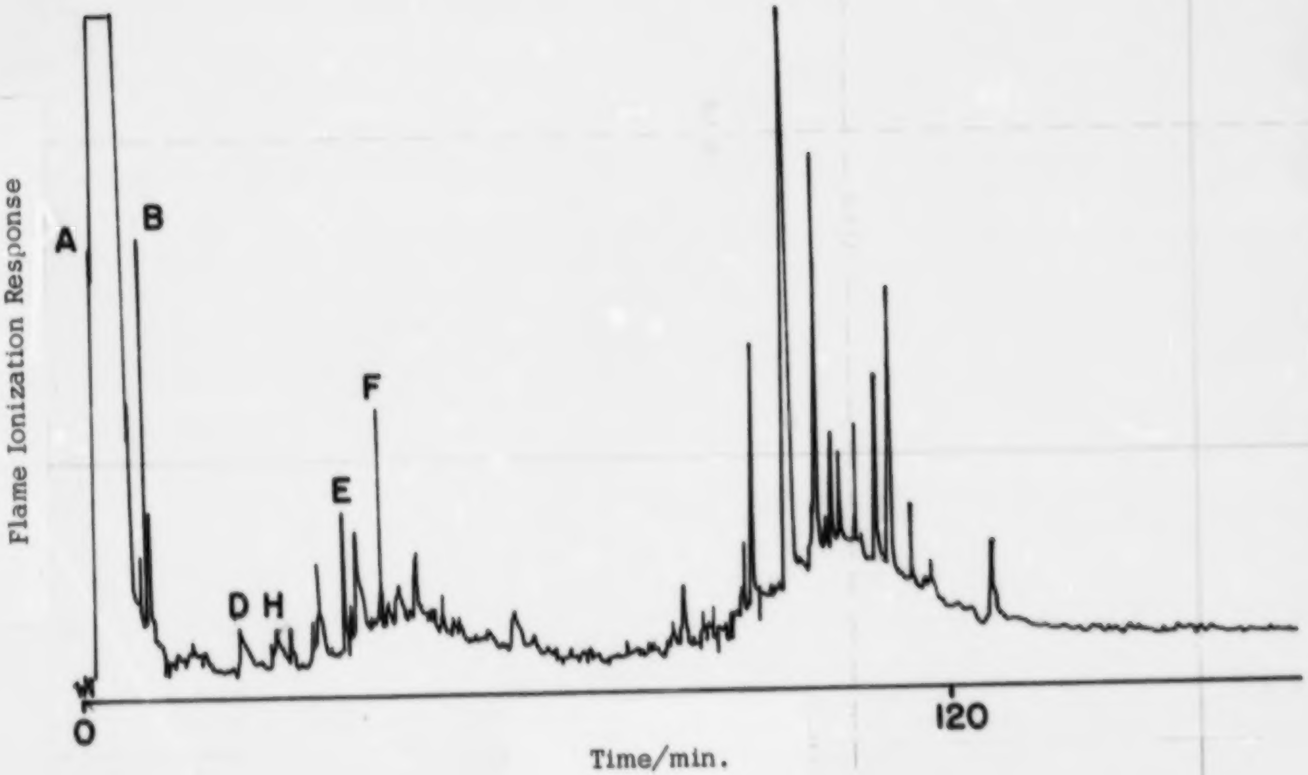

Figure 13. Chromatogram of Port St. Joe, Florida, wastewater concentrate: methylated.

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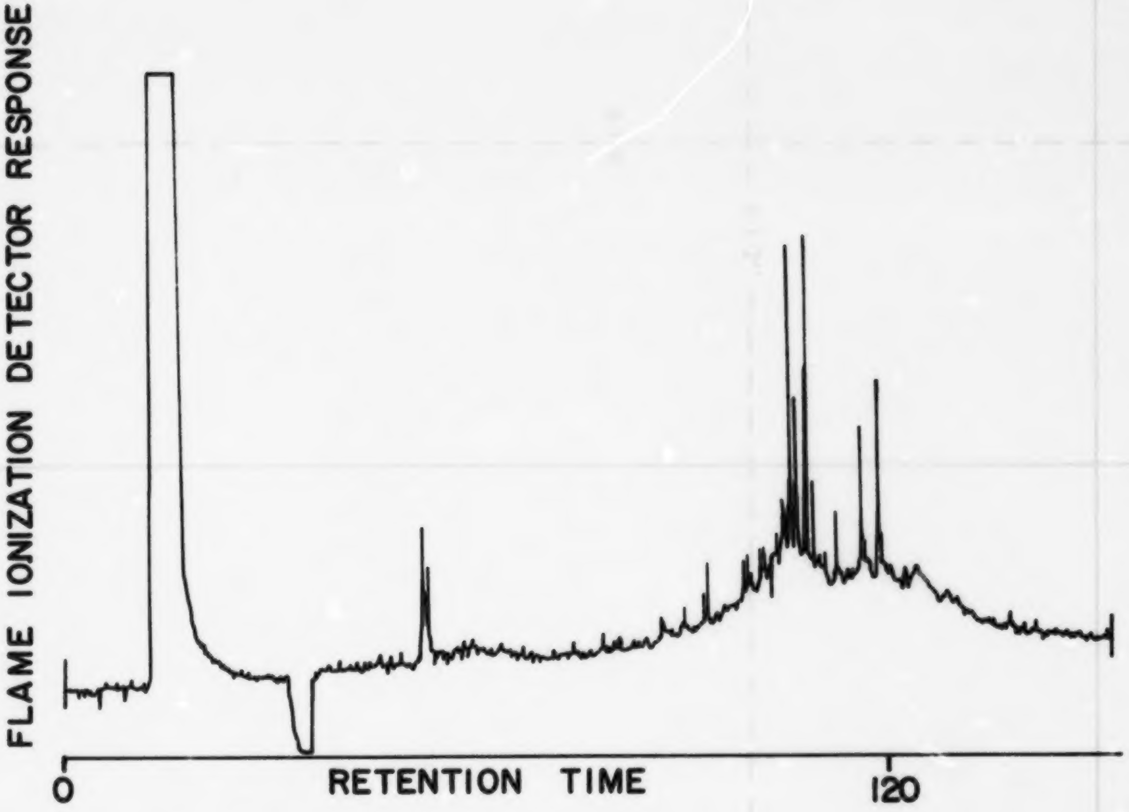

Figure 14. Chromatogram of Port St. Joe, Florida, ozonated wastewater.

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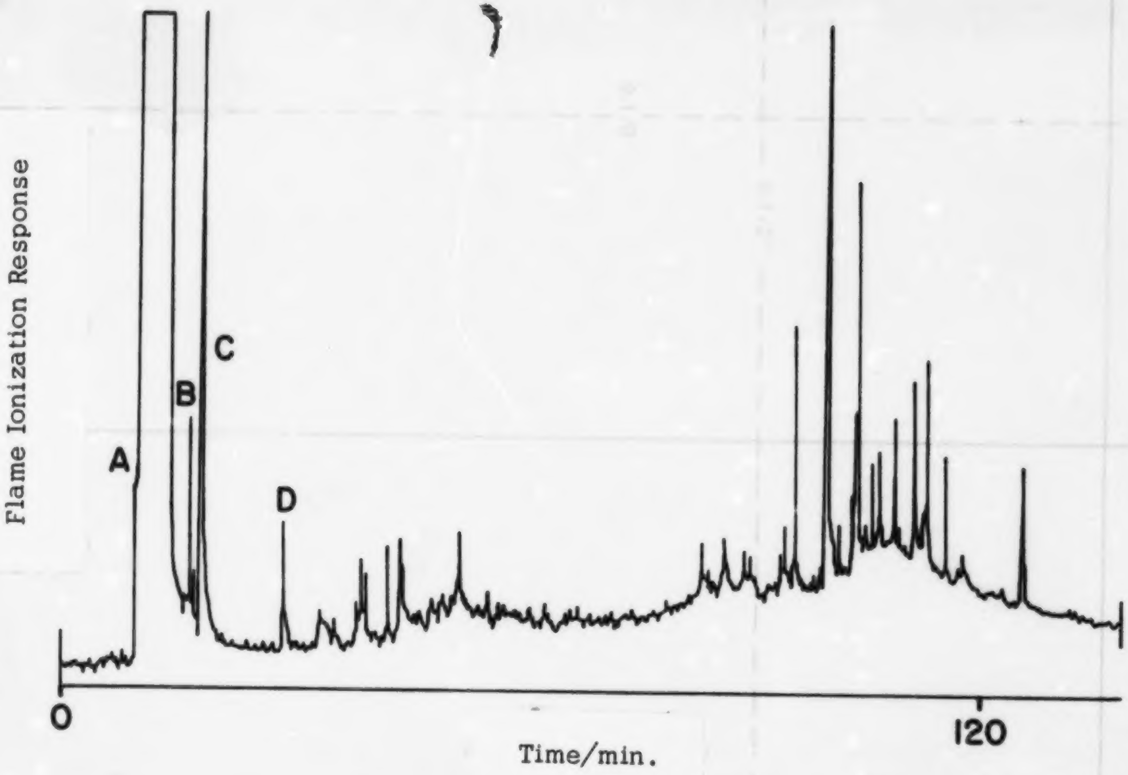

Figure 15. Chromatogram of Port St. Joe, Florida ozonated wastewater: methylated.

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TABLE 8. COMPOUNDS IDENTIFIED IN WASTEWATER FROM A SPECIALTY CHEMICAL COMPANY (See Figures 16, 17, and 18).

Peak Designation*	Retention Time/min	Compound
1	1.1	+
2	2.1	Ferrocene
3	3.1	Chlorotoluene isomer
4	3.4	Biphenyl
5	3.8	Diphenylmethane
6	4.2	2,6-Di-t-butyl-p-cresol (BHT)++
7	5.1	2-Methoxynaphthalene
8	6.1	2-Ethoxynaphthalene
9	6.7	+
10	7.3	+
11	7.9	+
12	8.4	Dicyclohexylbenzene isomer
13	8.7	Dicyclohexylbenzene isomer
14	8.9	Dicyclohexylbenzene isomer
15	9.4	2-Bromo-6-Methoxynaphthalene
16	9.7	o-Terphenyl
17	10.8	Octadecylbromide
18	11.2	+
19	11.7	+
20	12.9	2-Acetyl-6-Methoxynaphthalene
21	13.5	2-Propionyl-6-Methoxynaphthalene
22	14.0	+
23	21.9	d,1-2-(6-Methoxy-2-naphthyl) propionic acid
A#	0.9	Benzaldehyde
B#	1.2	Phenylacetaldehyde
C#	2.6	Benzyl alcohol
D#	3.9	+
E#	4.7	2-Benzoxazolone
F**	0.7	Bromoform

* Numbered peaks are from Figure 16

+ This compound not identified

# This peak is from Figure 17: ozonated water

** This peak is from Figure 18: chlorinated water

++ BHT is an antioxidant component of the solvent used in these experiments.

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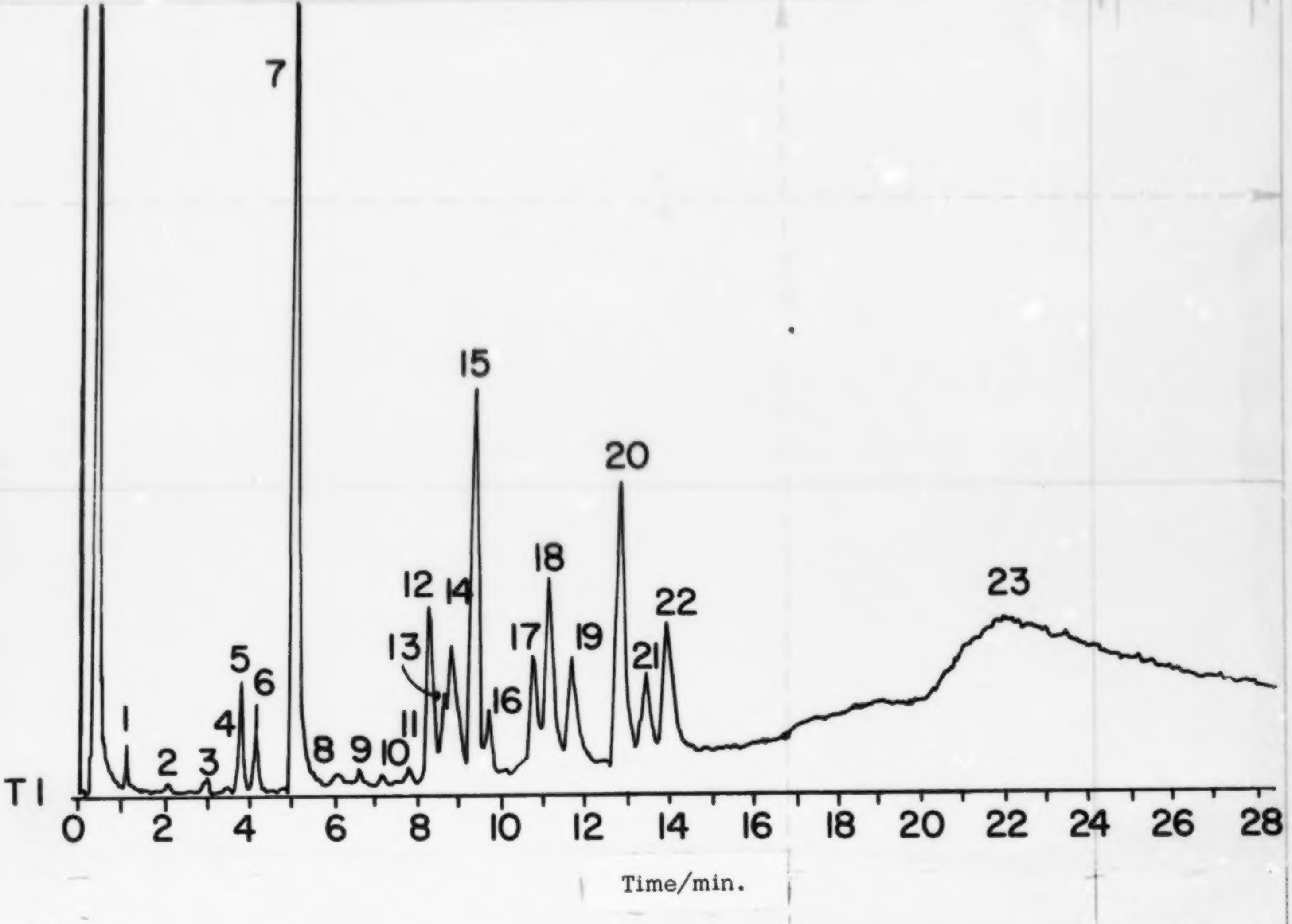

Figure 16. Chromatogram of specialty chemical company wastewater extract.

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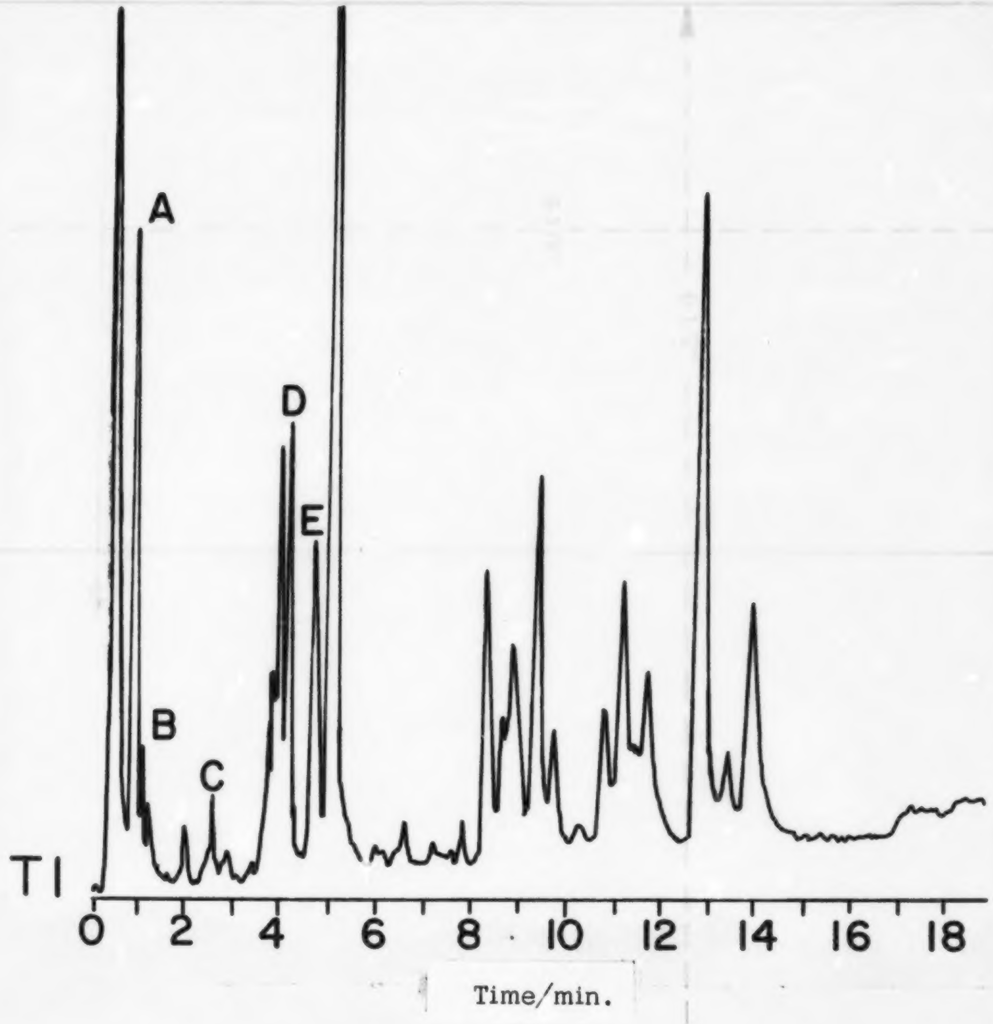

Figure 17. Chromatogram of specialty chemical company wastewater extract: ozonated.

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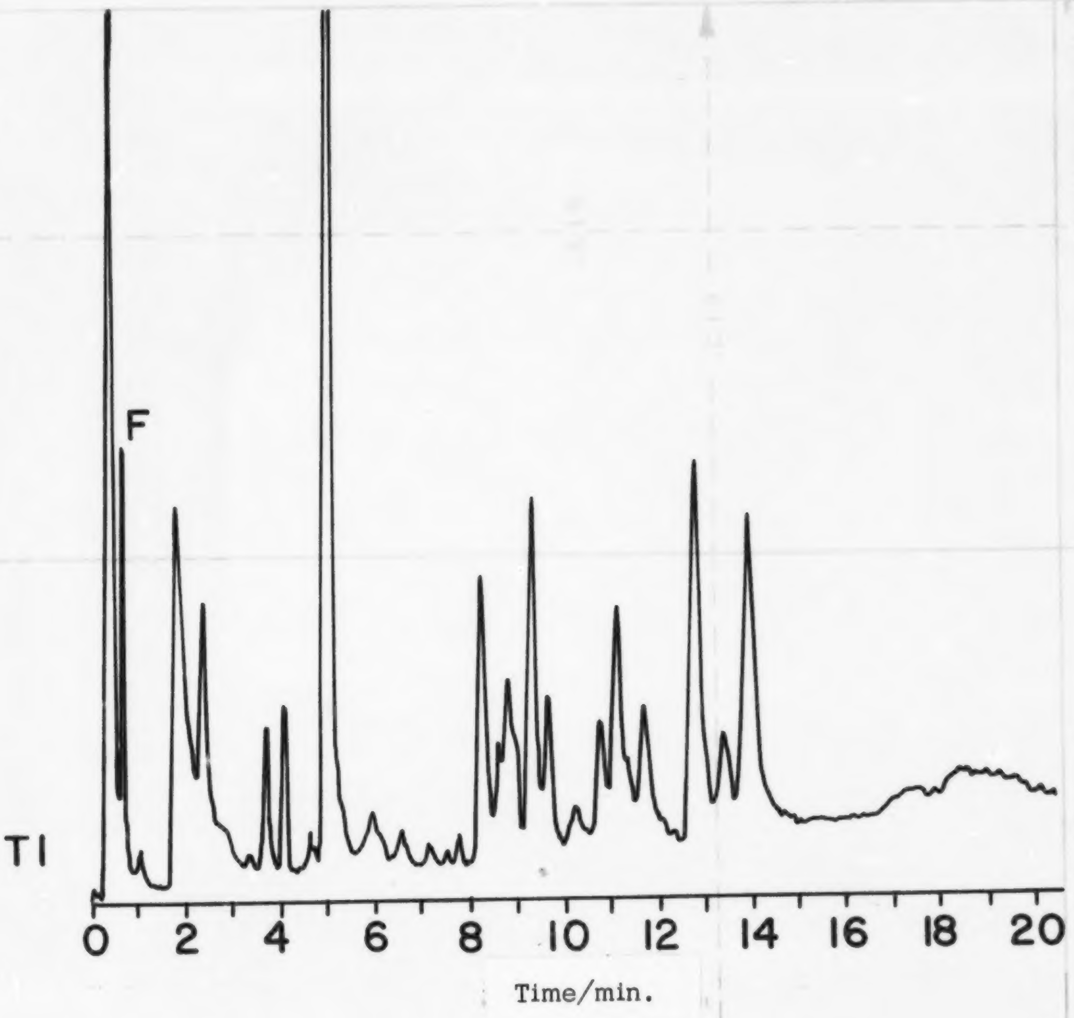

Figure 18. Chromatogram of specialty chemical company wastewater extract: chlorinated.

Chemical Manufacturing Site

Various wastewater samples were obtained from a major chemical manufacturing facility at Denver, Colorado. The complexity of these samples is shown in the chromatograms given in Figures 19 and 20. These data were obtained from dichloromethane extracts of the sample. No attempt was made to identify all components in these samples; however, several classes of compounds have been determined and some individual compounds have been identified in order to select model compounds for laboratory study. The list in Table 9 indicates these compound classes and identifications. For other aliquots of these samples, volatiles were purged and trapped in Tenax-GC. Data are presented in Figures 21 and 22 and Table 10.

TABLE 9 INDIVIDUAL COMPOUNDS IDENTIFIED

Figure #	Retention Time/Min	Compound
19	3.4	1-methylnapthalene
	3.9	Tetrachlorobenzene isomer
	7.7	Triethylene glycol
	11.6	*p*-chlorophenylomethyl sulfone
	12.4	*iso*-butylphthalate
20	1.7	Trimethylphosphate
	9.3	*p*-chlorophenylmethyl sulfoxide
	10.6	*p*-chlorophenylmethyl sulfone

EVALUATION OF RESULTS

Chemical Changes Caused by Ozonation of Wastewater

Studies of ozonized wastewater from the UTSD plant show that several of organic compounds are formed in the disinfection step of the treatment process. The compounds most commonly found to result from wastewater ozonation are aldehydes and alkanes. Although the data from the treatment plant do not

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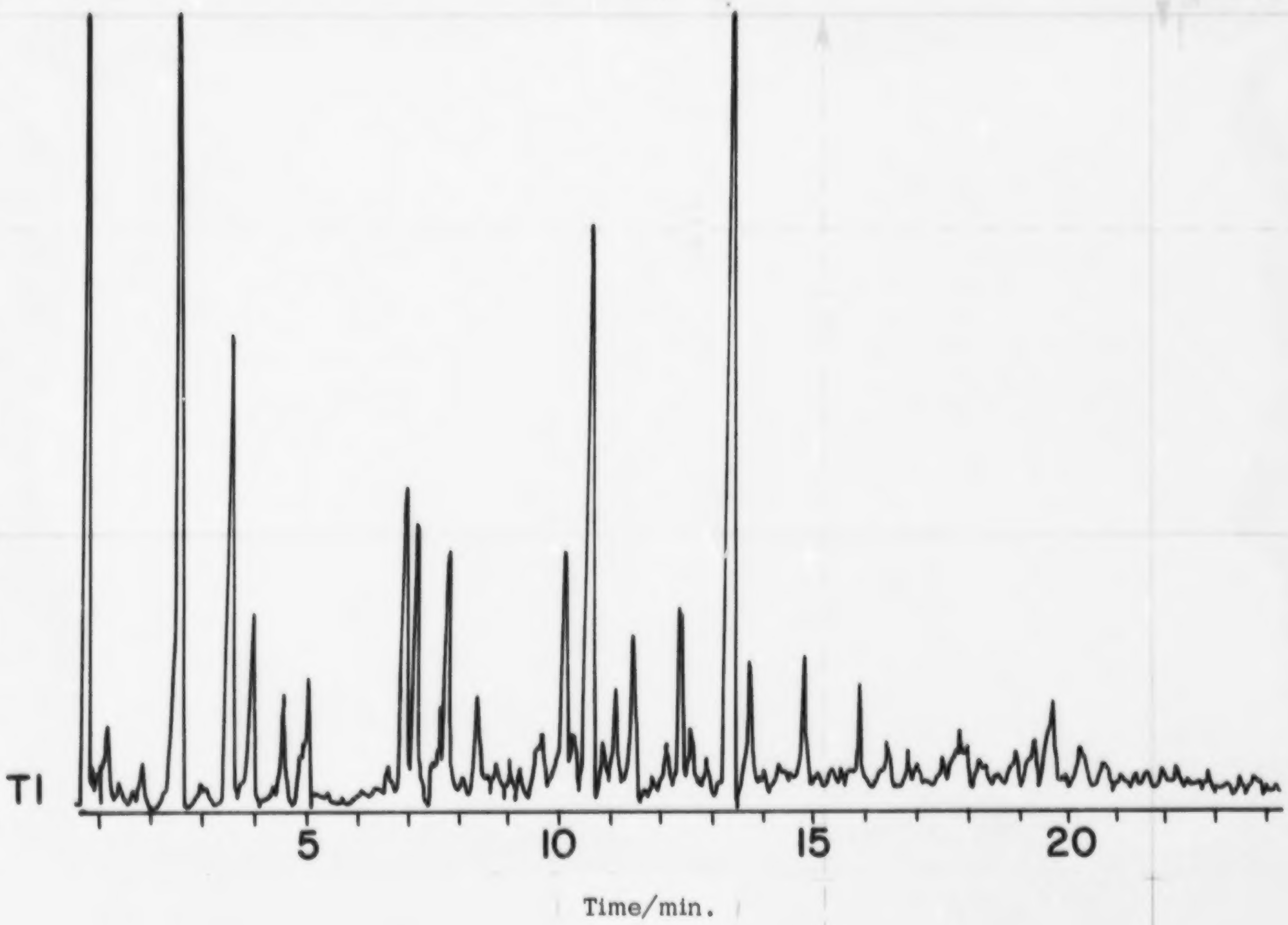

Figure 19. Mass-resolved chromatogram of a wastewater extract from a chemical facility disposal well.

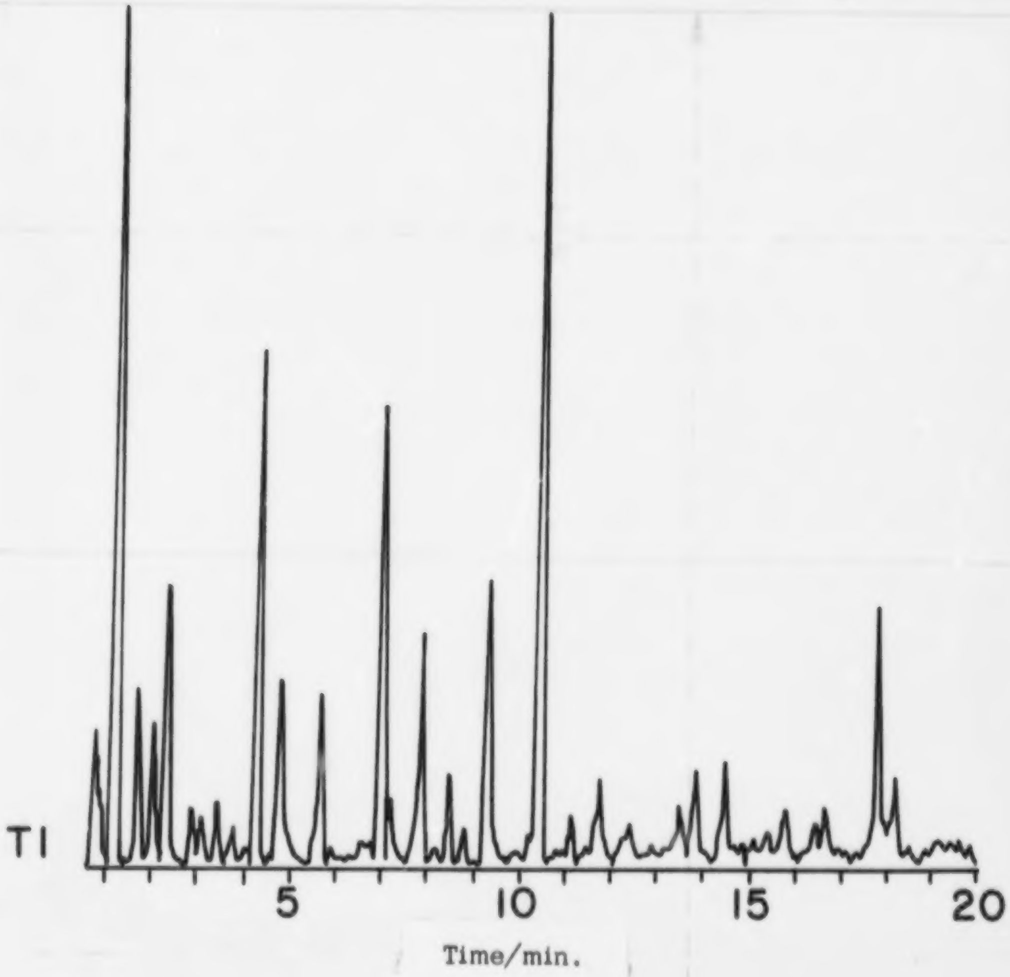

Figure 20. Mass-resolved chromatogram of a wastewater extract from a chemical facility disposal lake.

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TABLE 10. VOLATILE COMPOUNDS IDENTIFIED IN WASTEWATER FROM A CHEMICAL MANUFACTURING FACILITY

Figure #	Retention Time/Min	Compound
21	5.9	Cyclopentadiene
	6.6	Chloroform
	7.0	1,2-Dichloroethane
	7.2	Benzene
	8.0	C_7H_8 isomer
	8.7	Dimethyl disulfide
	9.4	Toluene
	9.8	C_7H_8 isomer
	10.5	Tetrachloroethylene
	12.7,13.4	Dimethylbenzene isomer
	13.8,14.2 18.0,18.4	$C_{10}H_{12}$ isomer
22	6.6	Chloroform
	7.2	Benzene
	9.4	Toluene

indicate the precursors of these compounds, laboratory ozonation of unsaturated organic acids, which have been shown to be present in domestic water at appreciable concentrations, shows that similar products are formed. Details of these reactions are discussed in a later section of this report. It should be noted here, however, that aldehydes, which are present in ozonized wastewater, also are destroyed by ozone to yield organic acids and alkanes. However, since no residual ozone was present in the UTSD effluent and aldehyde were found in the treated water, it is apparent that ozone treatment was insufficient to destroy as many ozonic species as possible. However, it must be remembered that ozone doses used to treat this water weren't adequate to

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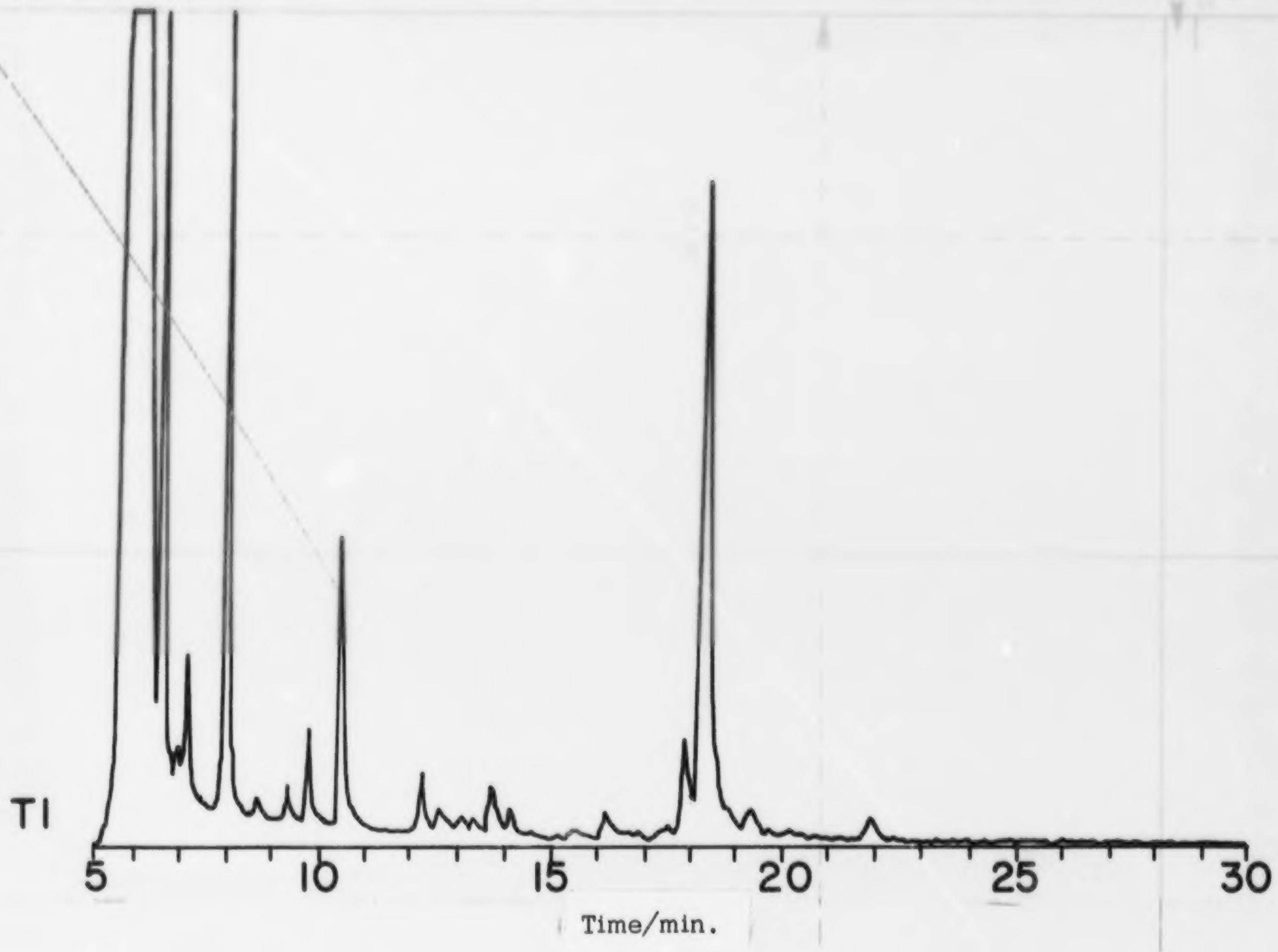

Figure 21. Chromatogram of wastewater volatiles from a chemical facility disposal well.

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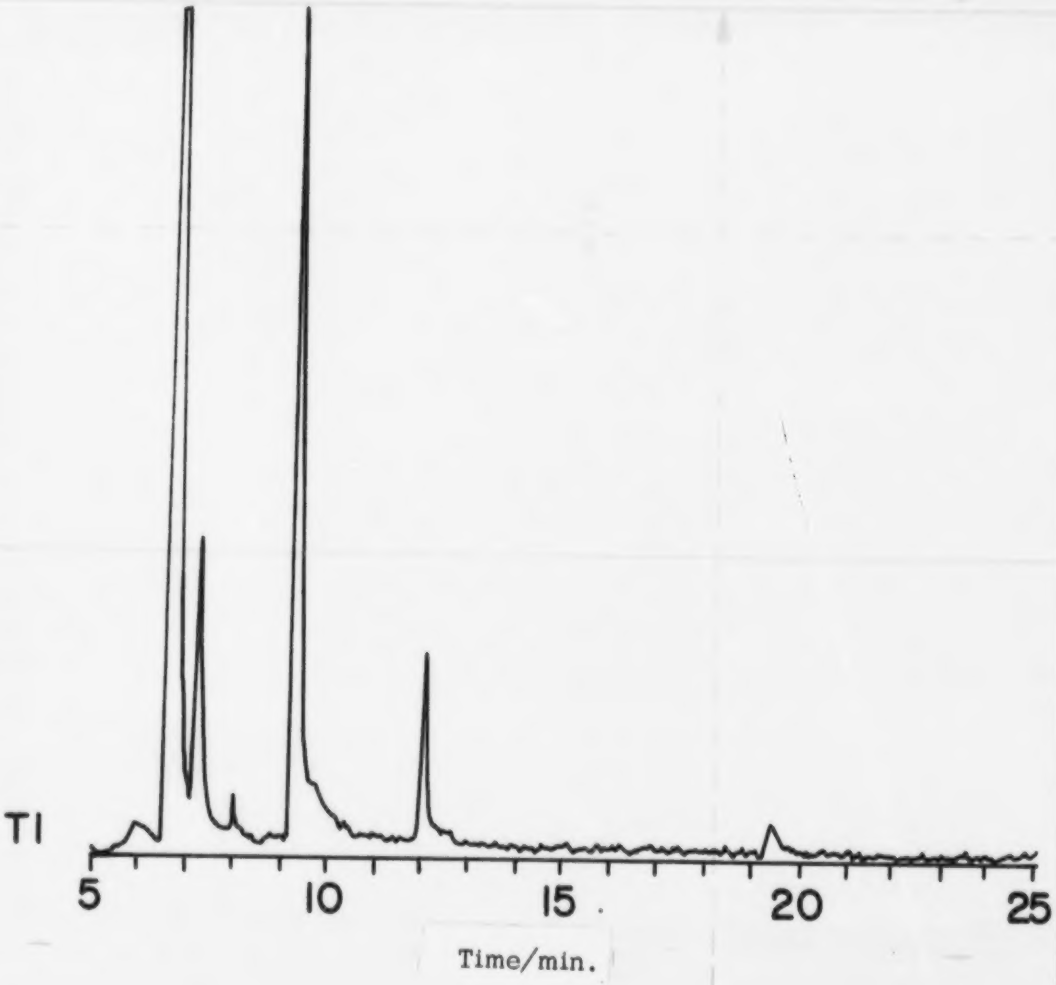

Figure 22. Chromatogram of wastewater volatiles from a chemical facility disposal lake.

effect disinfection and that there was no requirement to meet all chemical ozone demand in this system. As yet, no rationalization is apparent for the decrease in organic esters as indicated by a comparison of Figures 7 and 8. The effect of ozone on these compounds requires further study.

Laboratory studies, as discussed in another section of this report, have shown that the following classes of compounds are susceptible to treatment by ozone: xanthines, purines, pyrimidines, unsaturated hydrocarbons, unsaturated fatty acids, aldehydes, alcohols, sulfides, sulfoxides, and phosphonate esters. The products of such reactions include aromatic and aliphatic aldehydes, acids, and their derivatives, saturated hydrocarbons, heterocycles, including triazine derivatives and imidazoline derivatives, and sulfones. Other classes of compounds are somewhat refractory to ozone treatment as shown from samples treated in the UTSD plant and in the laboratory. These include: aromatic hydrocarbons, chlorinated pesticides, chlorinated aliphatic and aromatic hydrocarbons, saturated hydrocarbons, aldehydes (partially oxidized), acids, esters (including phthalate esters), sulfones, and phosphonate esters (partially oxidized). These results are based, in part, on data from experiments in the laboratory using relatively pure water as the solvent. Unlike the conditions in the treatment plant where biological organisms and a complex matrix of organic materials exists, the solutions used in the laboratory studies are relatively uncomplicated. However, assuming that sufficient ozone is present, the destruction of the reactive materials listed is possible within the constraints of their relative reactivities and other potentially more reactive species in a particular wastewater sample.

Mechanism of Reaction and Long-lived Intermediates

Laboratory studies designed to determine possible reaction mechanisms and long-lived intermediates have been made for the xanthine and unsaturated fatty acid model compounds. Certain compound types in the reactions can be considered to be long-lived intermediates. For example, aldehydes are both produced and destroyed by ozone and these are truly long-lived intermediates that can be isolated from reaction mixtures. Moreover, we have obtained sketchy evidence that organic peroxides and hydroperoxides may be long-lived intermediates. This is indicated by the persistence of oxidizing power in ozonized samples for several hours. Studies of the residual oxidizing power of ozone itself has led to the conclusion that the half-life of ozone in water is highly dependent upon organic and inorganic components in water. Such components appear to facilitate the decomposition of ozone which may result in the formation of peroxides and hydroperoxides.

Relatively long-lived free-radicals appear to be reaction intermediates in the ozonation of alkanes and aldehydes in water. This is shown by the formation of alkane dimers from these reactions (e.g., hexadecane is formed from ozonation of *n*-nonanal *via* *n*-octyl radical). In addition, the ozonation

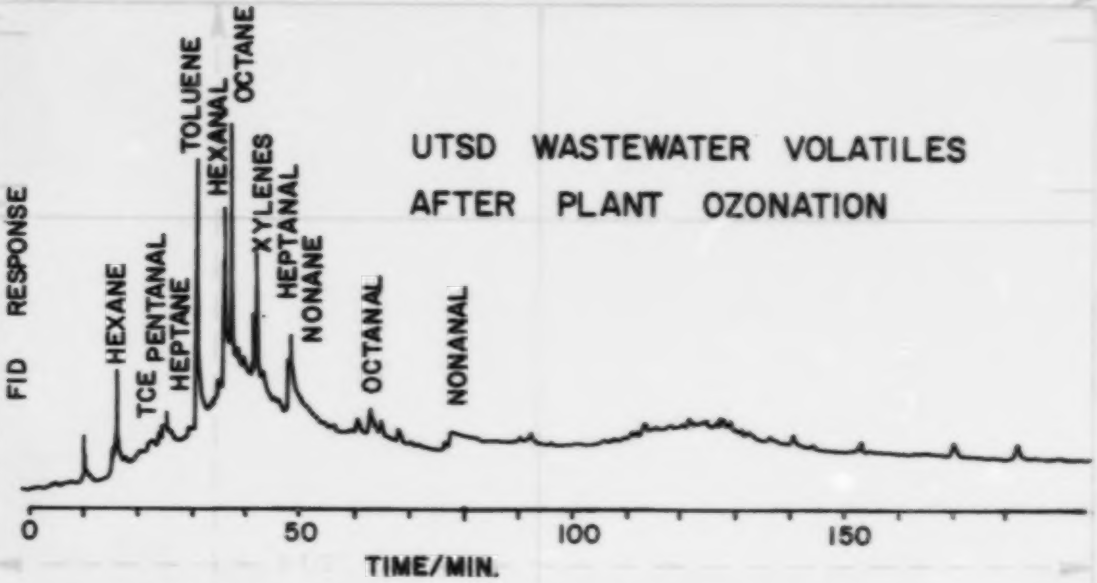

Figure 23. UTSD wastewater volatiles after plant ozonation.

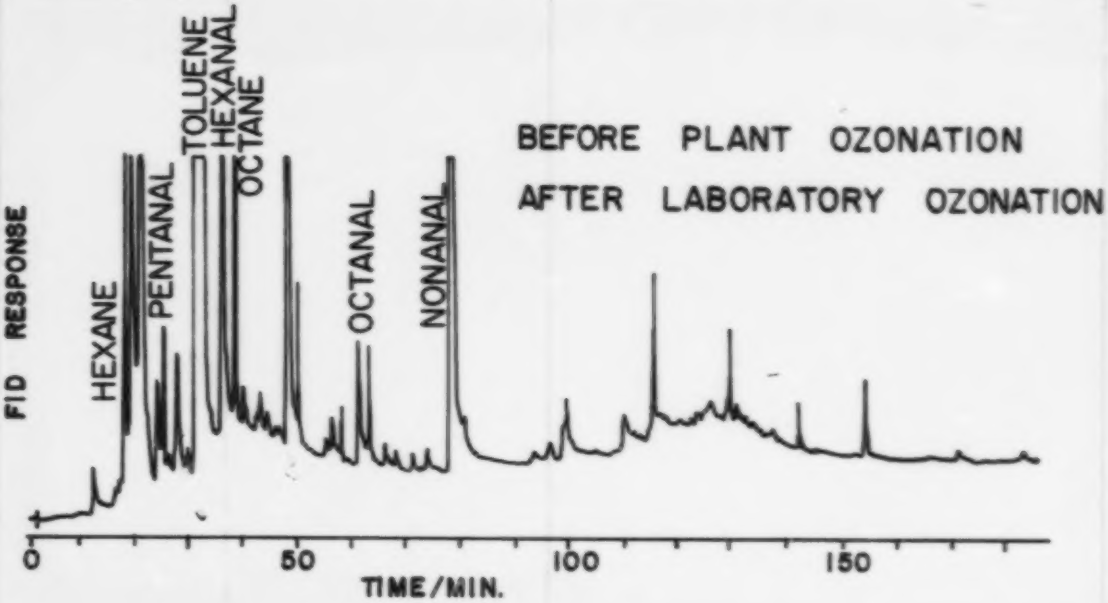

Figure 24. UTSD wastewater volatiles before ozonation at the treatment plant and after ozonation in the laboratory.

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of xanthine derivatives yields intermediate N-formylamines (amides) which are further oxidized upon prolonged ozone treatment.

Additional details concerning these long-lived intermediates are presented in following sections of this report.

Ozone Contact Time

Only limited studies have been made to determine the effect of ozone contact time with respect to the chemistry which occurs in wastewater. The Port St. Joe, Florida, samples as given in Figures 12-15 show that substantial changes occur for short contact times when ozonation takes place under laboratory conditions. At longer contact times in laboratory experiments, even greater change is observed in these samples, but such data do not necessarily relate to treatment plant conditions where ozone doses may not be sufficient to meet high chemical ozone demand. Many volatile compounds are lost from solution in these experiments because they are purged from the water sample during the ozonation process. (The same phenomenon probably occurs in the UTSD treatment plant, but to a lesser extent than in these laboratory experiments).

Another experiment has shown that the contact time and ozone doses used in laboratory experiments have a greater effect on the chemistry of wastewater than do UTSD plant conditions. An unozonized sample of UTSD water was treated with ozone in the laboratory (see Figures 23 and 24). Qualitatively, the same changes were observed in the volatile constituents as in ozonated wastewater obtained from the UTSD facility; however, greater concentrations of all components were observed including the high molecular weight alkanes found in plant ozonated samples. These alkanes, presumably the dimers of the radical species produced from the ozonation of unsaturated materials, were present at a concentration at least ten times greater than found in UTSD treated wastewater. These data indicate that substantial changes occur in the organic composition of wastewater samples when total chemical ozone demand is approached.

Effect of Chloride Ion Concentration

High concentration of chloride ion in ozonation mixtures does not cause production of chlorine-containing products.

Comparison with Chlorine Treatment

The data presented in Figures 16-18 and Table 8 for samples obtained from the effluent of a specialty chemical concern indicate that ozonation causes many new compounds to be formed, thus indicating that some of the organic material present is susceptible to ozone treatment. Treatment with

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chlorine, however, yields only one new compound in appreciable concentration. This water is heavily contaminated with bromide ion and brominated products are expected upon chlorine treatment, but it is interesting that no other new compounds were observed in this treatment.

SECTION 4

OZONATION OF MODEL COMPOUNDS

INTRODUCTION

The ozonation of several model compounds has been studied to determine possible end products when substrate species similar to these compounds are present in water prior to ozone treatment. The model compounds studied include those found in UTSD samples as well in samples from other wastewater sources which are likely candidates for ozone treatment.

Caffeine is a common constituent of domestic wastewater systems and the study of its ozonation is representative of the chemistry that occurs when a common complex molecule is subjected to ozone treatment. Although caffeine was identified in UTSD water prior to ozonation, none of the products of caffeine ozonation were found in UTSD samples after treatment. However, the analytical methods used in the UTSD studies precluded routine analyses of these compounds because they are less volatile than necessary for the purge and trap method. There is, though, no reason to expect that the same products of caffeine ozonation would not be found in ozonated wastewater if sufficient ozone is used to react first with other more reactive biological and chemical species.

Our study of the ozonation of aldehydes and fatty acids also is directly related to species found in UTSD samples. Fatty acids and other relatively long-chain unsaturated species are present in any domestic wastewater and are easily oxidized by ozone, as are aldehydes, which themselves are products of ozonation of unsaturated organic compounds.

Laboratory studies of Lignosite®, *p*-chlorophenylomethyl sulfone, diisopropylmethylphosphonate, and dicyclopentadiene, indicate the chemistry expected when these compounds are subjected to treatment with ozone. These and related materials are possible candidates for treatment with ozone in various industrial applications.

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XANTHINE AND PYRIMIDINE DERIVATIVES

A chloroform extract of one of the first UTSD samples collected in June, 1976 was shown by GC/MS analysis to contain caffeine (Figure 25). (Due to excessive background other compounds in this sample could not be identified). At the time this sample was obtained the ozonation basin was not operational and no comparison with samples ozonated in the treatment plant could be made. However, a literature search indicated that the aqueous ozonation chemistry of caffeine, begun by Leipen[26] in 1890, was not complete and offered interesting possibilities for a model compound study of a component contained in the influent to the UTSD facility.

Leipen[26] reported that ozonation of an aqueous solution of caffeine (1) produced dimethylparabanic acid (2), methylamine (3), ammonia, and carbon dioxide (Figure 26). Others have shown that dimethylparabanic acid is the stable endproduct from oxidation of caffeine under many experimental conditions, but it was not until 1882 that Emil Fischer determined its structure[27]. Subsequently, several other workers have studied oxidations of caffeine, and in all cases dimethylparabanic acid was the only non-gaseous product reported[28].

Caffeine (1), theophylline (4), and theobromine (5), are methylated derivatives of xanthine (compound 6, see Figure 27) which are found in the plant kingdom throughout the world. These substances are central nervous system stimulants and aqueous plant extracts containing these materials have been consumed by humans since the earliest times. The most common modern day beverages include coffee, tea, cocoa, and cola-flavored drinks. It seems likely that the caffeine in the UTSD wastewater system originates from the disposal of the residue from one or more of these beverages.

The xanthines are derivatives of the purine (7) ring system (xanthine is 2,6-dioxopurine, see Figure 27). In turn, the purine ring system can be considered a derivative of the pyrimidine (8) ring system. These two ring systems form the nucleaus of five other important biological compounds: adenine (9), guanine (10), uracil (11), thymine (12), and cytosine (13, see Figure 28). These five compounds constitute the major nitrogenous bases present in nucleic acids and provide the code sequence responsible for storage and transmission of genetic information.

The ozonation of the model compounds caffeine (1), 1,3-dimethyluracil (14), and 1,3-dimethylthymine (15, see Figure 29) was conducted in this study in order to elucidate the ozonation chemistry of this important class of heterocyclic compounds. It was anticipated that the ozonation reactions of these substances would accurately depict those of other purine and pyrimidine bases. The direct study of the nitrogenous bases urancil and thymine (see Figure 28 was complicated by the non-volatility of the resulting ozonation

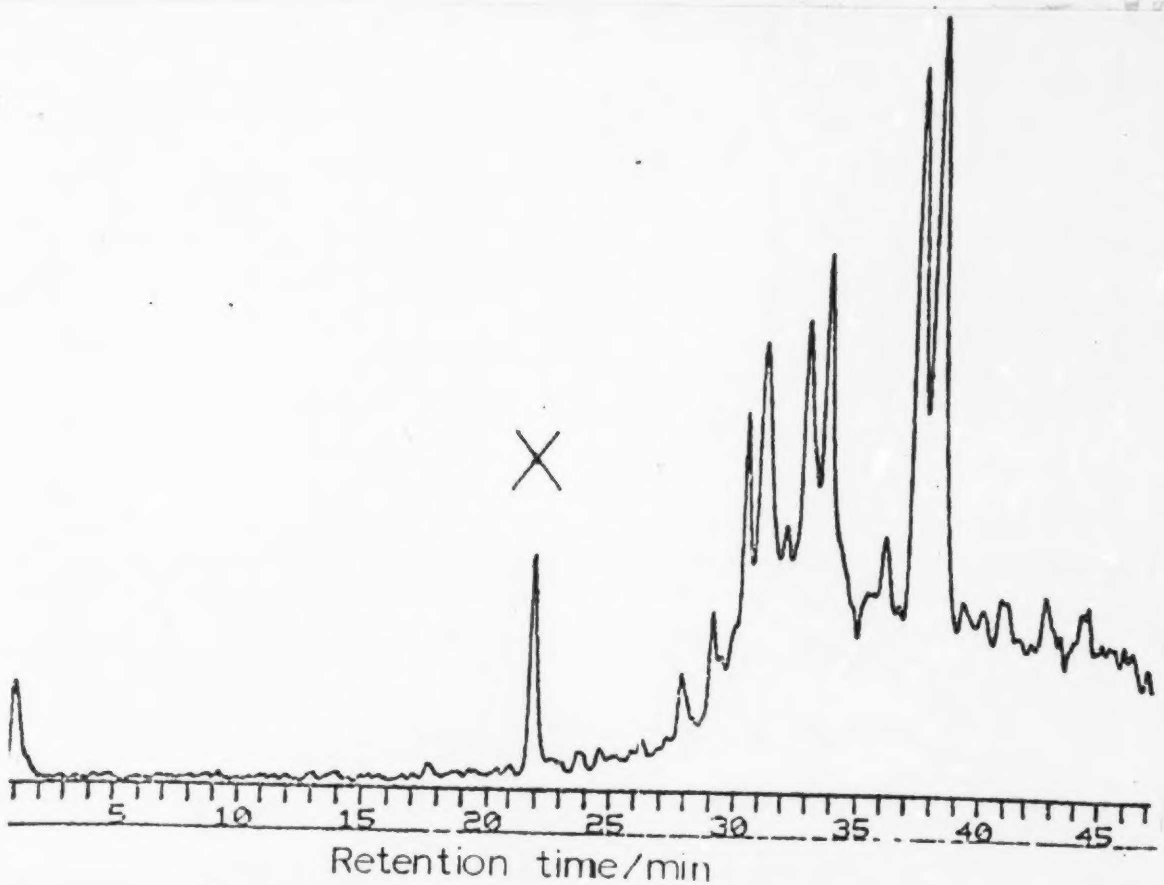

Figure 25. Chromatogram of UTSD chloroform extract X indicates peak identified as caffeine by MS and retention time.

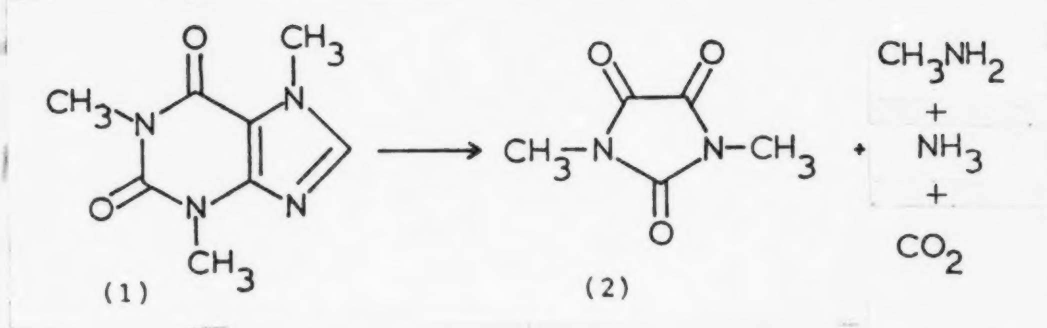
O
CH3
CH3
N
N
O
N
N
CH3
(1)
O
O
CH3–N
N–CH3
O
(2)
+
CH3NH2
+
NH3
+
CO2

Figure 26. Caffeine (1) ozonation products (1890).

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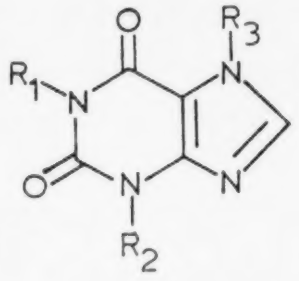
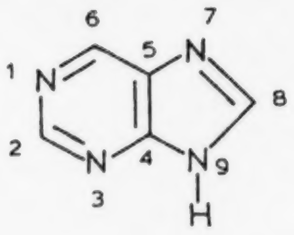


(1) $R_1 = R_2 = R_3 = CH_3$

(4) $R_1 = R_2 = CH_3$, $R_3 = H$

(5) $R_1 = H$, $R_2 = R_3 = CH_3$

(6) $R_1 = R_2 = R_3 = H$



Purine

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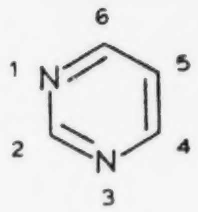

Pyrimidine

(8)

Figure 27. Relation of xanthine to purine and pyrimidine ring systems.

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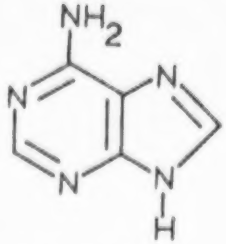
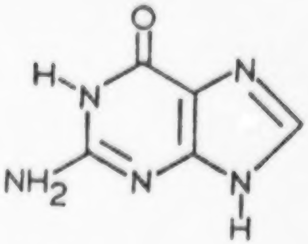
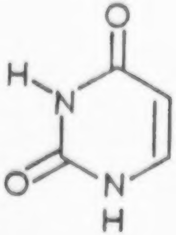


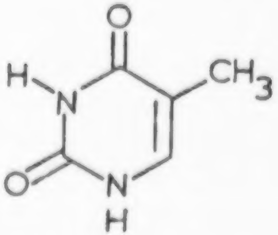
(9) Adenine



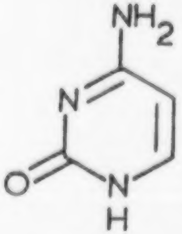
(10) Guanine



(11) Uracil



(12) Thymine



(13) Cytosine

Figure 28. Major nitrogenous bases found in nucleic acids.

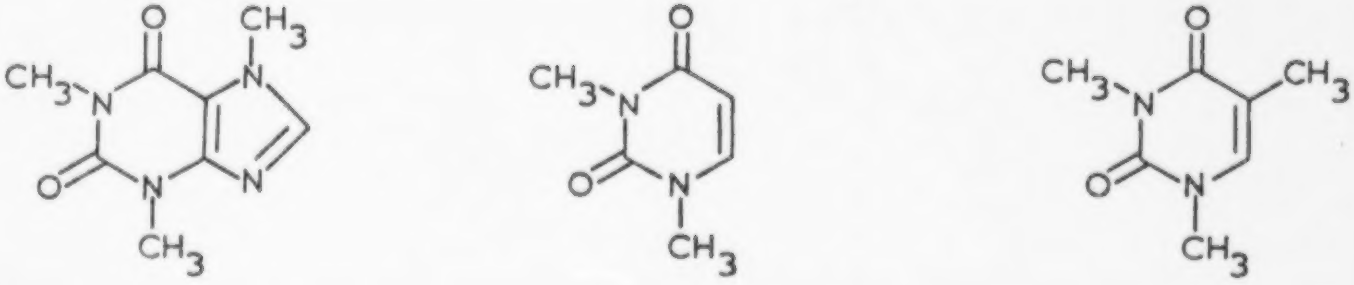


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Figure 29. Methylated derivatives containing the purine or pyrimidine nucleus.

products. Caffeine and the methylated uracil and thymine derivatives produced volatile ozonation products which were readily analyzed by GC/MS techniques. The methylated uracil and thymine derivatives, rather than the natural non-methylated materials (see Figure 28), were studied for this reason. The validity of this approach was supported by the fact that the ozonation characteristics of the uracil versus 1,3-dimethyluracil (14) reaction were found to be similar. In each case a parabanic acid was formed. Further study is still needed in this area. The use of high performance liquid chromatography, which was not available to us, would facilitate the analysis of non-volatile ozonation products. The preparation of volatile derivatives of these ozonation products should represent another approach to this volatility problem. However, this approach met with little success and thus suitable derivatization procedures were not found in the examples studied.

Ozonation of Caffeine: General Reaction Features

The initial ozonation of caffeine was conducted in water solution at high concentration. A concentration range of 6661333 ppm caffeine was employed to facilitate product isolation and identification. The results obtained in these experiements were identical to those obtained in later experiments when the initial caffeine concentration was only 2 ppm. In each case the same product mixture was produced.

Upon ozonation, caffeine is rapidly destroyed by ozone and at least seventeen discernible products result, as determined by gas chromatography. The initial ozonation reaction was conducted at a caffeine concentration of 3.4×10^{-3}M (666 ppm) and a reaction time of 90 minutes at 20° C. The solution was allowed to react with an ozone-oxygen stream introduced at a flow of 600 mL/min (17 mg O_3/minute). After 20 minutes all of the caffeine was converted to two major products and many minor products. The two major products constituted about 50% of the product mixture. A gas chromatography/mass spectrometry (GC/MS) experiment conducted of the product mixture gave the total ion chromatogram shown in Figure 30. The product ratio, molecular weight, and gas chromatographic retention time are shown in Table 11. The relative product ratios were determined by flame ionization detection (FID) and were not corrected for differences in detector response. The mass spectrum for each component is tabulated in Table 12.

The amount of ozone consumed per mole of caffeine destroyed was determined by a procedure described by Smith, Greenwood, and Hudrlik[29]. The experiment demonstrated that 4.2 moles of ozone were consumed by each mole of caffeine.

The effect of ferric and ferrous ion on the oxidation of caffeine was examined and was found to be rather dramatic. The results are shown in Table 13. It was found that as the ferric or ferrous ion concentration increases the relative yields of 1,3-dimethyl-5azabarbituric acid and 1,3-dimethyl-oxonic acid methyl-amide increases at the expense of dimethlparabanic acid. It is probable that ferrous is oxidized to ferric by the ozone. No difference was noted in product ratio when either ferric or ferrous were used.

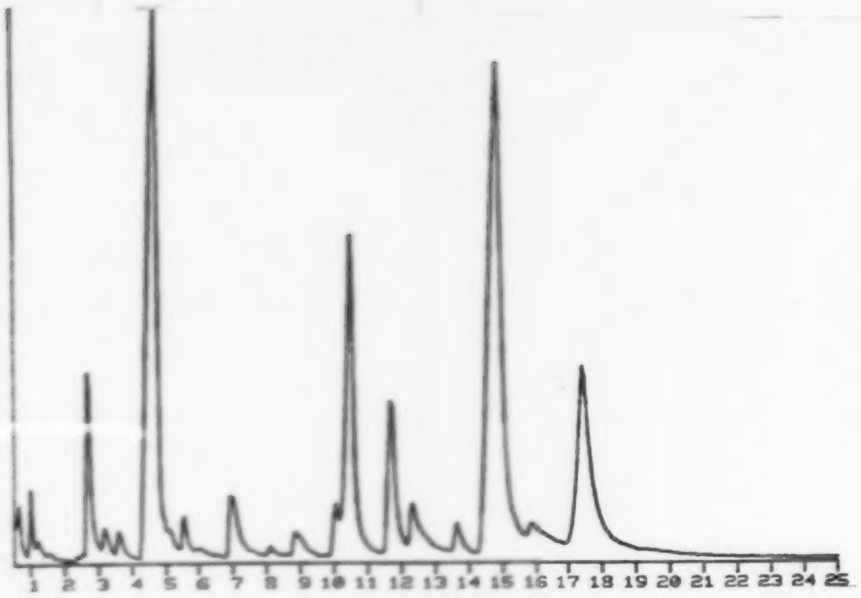

Figure 30. Total ion chromatogram of caffeine ozonation products.

TABLE 11. PRODUCTS FORMED FROM CAFFEINE OZONATION[a]

Retention Time (tr)	Mol. wt.	Relative Percent[b]
1.0 min	75	---
1.2 min	59	---
2.7 min	117	6.0
3.2 min	116	0.6
3.6 min	132	0.1
4.5 min	142	23.0
5.6 min	102	1.6
7.0 min	140	3.6
8.9 min	143	2.4
10.1 min	144	2.7
10.5 min	157	8.7
11.7 min	141	3.4
12.3 min	128	5.8
13.7 min	210	1.6
14.9 min	198	26.4
15.9 min	254	2.4
17.4 min	226	11.7

[a] Gas chromatography/mass spectrometry (GC/MS) was conducted according to conditions A listed in the experimental section.

[b] Relative percent yield was measured by FID response according to GC conditions B.

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TABLE 12. EIGHT PRINCIPAL PEAKS IN THE MASS SPECTRA OF CAFFEINE OZONATION PRODUCTS[a]

Mol Wt.	t_r (min)	1	2	3	4	5	6	7	8
75	1.0	44(100)	31(31)	46(26)	75(20)	45(20)	29(15)	------	------
59	1.2	54(100)	30(74)	31(72)	29(54)	28(50)	32(44)	------	------
117	2.8	58(100)	89(20)	28(13)	59(9)	15(9)	177(6)	29(6)	------
116	3.2	58(100)	30(44)	116(33)	44(32)	59(29)	88(8)	------	------
132	3.6	58(100)	30(80)	28(31)	59(30)	45(28)	57(26)	44(30)	132(20)
142	4.5	142(100)	58(80)	56(60)	57(48)	70(19)	114(10)	84(5)	------
102	5.6	58(100)	31(65)	32(56)	30(45)	44(43)	59(30)	102(10)	------
140	7.0	42(100)	140(84)	55(64)	83(27)	28(27)	82(23)	------	------
143	8.9	58(100)	143(82)	28(21)	29(12)	44(9)	45(9)	42(9)	------
144	10.1	59(100)	58(87)	42(70)	116(51)	144(23)	88(22)	56(18)	127(7)
157	10.5	157(100)	58(74)	56(51)	57(31)	70(22)	44(11)	100(9)	129(8)
141	11.7	141(100)	42(98)	29(37)	56(30)	84(30)	41(18)	29(12)	83(12)
128	12.3	56(100)	128(70)	57(61)	100(41)	43(34)	70(24)	44(22)	42(16)
210	13.7	42(100)	182(41)	56(34)	69(20)	167(11)	97(7)	210(5)	142(5)
198	14.9	56(100)	83(80)	44(44)	58(42)	170(38)	141(32)	198(31)	113(7)
254	15.9	42(100)	127(61)	56(33)	169(24)	70(24)	28(22)	128(19)	254(15)
226	17.4	83(100)	45(78)	30(37)	28(28)	156(24)	184(17)	141(17)	226(16)

[a] Isotope peaks are ignored

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TABLE 13. THE OZONATION OF CAFFEINE IN THE PRESENCE OF IRON

Product	0.0	0.125[a]	0.25[a]	0.5[a]	1.0[a]	1.0[b]
II	39%	35%	24%	10%	6%	6%
III	12%	14%	50%	20%	21%	22%
IV	49%	52%	56%	70%	70%	72%

[a] Fe (II) as $FeSO_4$
[b] Fe (III) as $Fe_2(SO_4)_3$

Major Products--

Dimethylparabanic acid is indeed a principal ozonation product of caffeine, but in our hands, it always ranked second in abundance to a component of molecular weight 198 (see Figure 30) tr = 4.5). Dimethylparabanic acid was isolated from a chloroform extract of the aqueous product mixture and purified by recrystallization from 95 percent ethanol. It was the only chloroform extractable product and was isolated in 27 percent yield.

The mass spectrum of dimethylparabanic acid exhibited a molecular ion (m/e 142) and fragmented by loss of 28 mass units (see Figure 31). Significant fragment ions were formed at m/z 56, 57, and 58. The infared spectrum showed the presence of a carbonyl absorption (1735 cm^{-1}). The proton magnetic resonance (^1H NMR) spectrum showed a singlet (3.20 ppm). The proton decoupled carbon magnetic resonance (^{13}C NMR) spectrum showed three resonances (24.9, 154.1, and 157.0 ppm). The absorption of 154.1 and 157.0 demonstrated that at least two carbonyl groups were present in the molecule. Based on these results and those of other workers[28] this compound was assigned the structure of dimethylparabanic acid. An authentic sample of dimethylparabanic acid was prepared by the reaction of 1,3-dimethylurea with oxalyl chloride and shown to be identical in every respect (MS, NMR, IR, and MP) with the caffeine ozonation product (Figure 32).

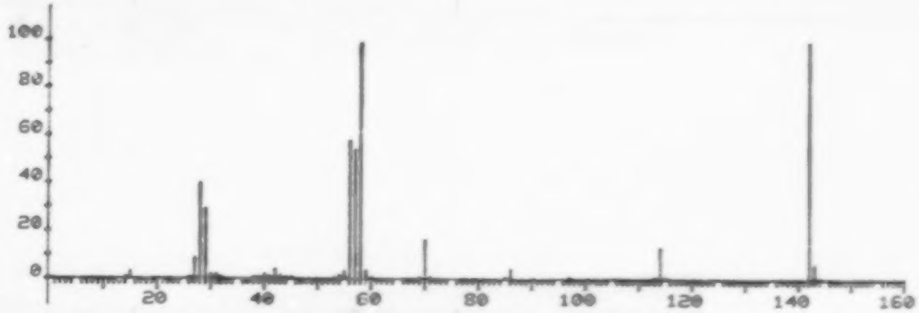
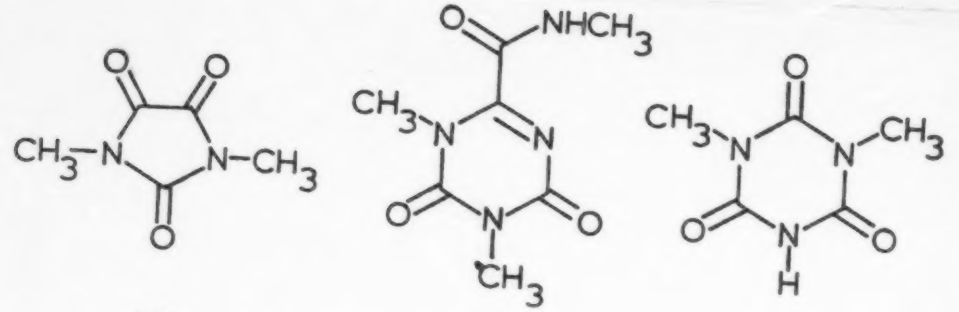


Figure 31. Mass spectrum of dimethylparabanic acid.



(2) (16) (17)

Figure 32. Caffeine ozonation products.

The major reaction product, compound 16 (tr = 14.9 minutes), was isolated and purified by column chromatography on silica gel. Several passes through the column were required to separate this component from unreacted caffeine and dimethylparabanic acid. By this technique compound 16 was ultimately obtained in a pure (chromatographically homogeneous) state. It crystallized upon evaporation of the eluting solvent to give a white solid (Mp 178-179°C).

The mass spectrum of compound 16 showed a molecular ion (m/e 198) which fragmented by loss of 28 and 57 mass units (see Figure 33). Significant fragment ions at m/e 56 and 83 were also formed. The infrared spectrum showed the presence of a carbonyl group (1670 cm^{-1}), a carbon-nitrogen double bond (1595 cm^{-1}), and a hydrogen attached to nitrogen (3160 cm^{-1}). The ^1H NMR spectrum showed three 3-proton signals, of which two appeared as singlets (3.42 and 3.78 ppm) and one appeared as a doublet (2.98 ppm). A broad one-proton resonance (7.80 ppm), attributed to an acidic proton attached to nitrogen, was also present. Addition of deuterium oxide to the ^1H NMR solution caused the disappearance of the resonance at 7.80 ppm and the collapse of the doublet (2.98 ppm) to a singlet. The infrared and NMR data are consistent with the presence of a N-methylamide moiety in the molecule.[30] The proton decoupled ^{13}C NMR spectrum revealed the presence of seven nonequivalent carbon resonances. The presence of three carbonyl groups in the molecule was demonstrated by the absorptions of 154.5, 156.1, and 159.1 ppm. The mass spectrum and NMR as well as elemental analysis demonstrated that the molecule has a formula $C_7H_{10}N_4O_3$. Based on this information three possible structures were proposed (see Figure 34).

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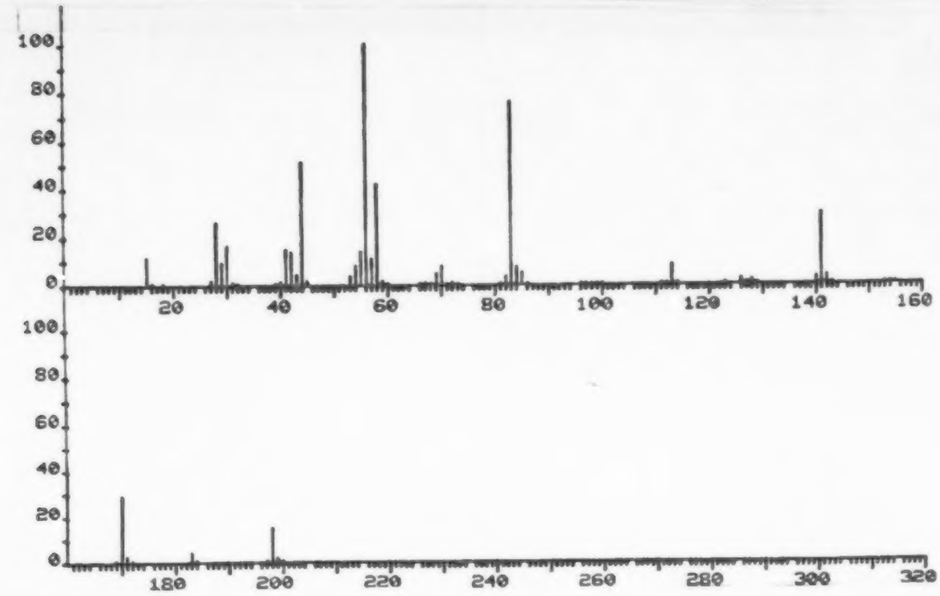
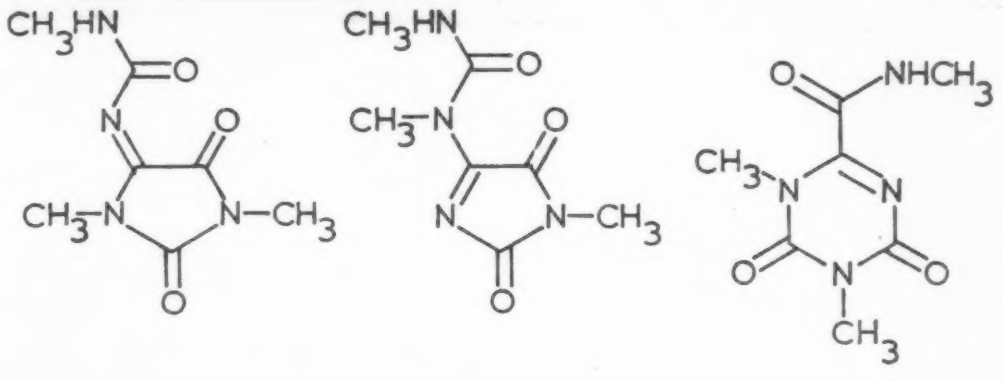


Figure 33. Mass spectrum of 1,3-dimethyl-oxonic acid methylamide (16).


CH3HN
O
N
O
CH3-N
N-CH3
O
CH3HN
O
CH3-N
O
N
N-CH3
O
O
NHCH3
CH3-N
N
O
N
O
CH3

A B C

Figure 34. Possible structures for compound 16.

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The structure shown for compound A was considered a likely possibility since it was rationalized that ozonation of the carbon-nitrogen double bond would produce dimethylparabanic acid. However, when compound 16 was allowed to react under the original caffeine ozonation conditions only starting material (compound 16) was recovered. Subsequently, an authentic sample of compound A was prepared by treatment of acetone cyanohydrin with methylisocyanate and comparative analysis showed compound A not to be the caffeine ozonation product.[31]

Examination of the analytical data on compound 16 did not permit us to distinguish this compound as having either structure B or C. Structure B was considered unlikely since all known examples of imidazolidines exist as trimers.[31] X-ray crystallography was employed and established[32] the structure of compound 16 as that shown as C in Figure 34. This compound has not been described in the chemical literature. It can be named, by Chemical Abstracts nomenclature, as 3,5-dimethyl03,4,5,6-tetrachydro-4,6-dioxo-1,3,5-triazine-2 carboxylic acid methylamide. This compound is a derivative of oxonic acid and will be referred to by the common name, 1,3-dimethyloxonic acid methylamide, for relative simplicity.

Compound 17 (tr = 10.5 min) was isolated by column chromatography through silica gel. This mass spectrum gave a molecular ion (m/e 157) which fragmented by loss of 57 mass units (see Figure 35). Significant ions were formed at m/e 56, 57, and 58 mass units. The ^1H NMR spectrum showed a six-proton singlet (3.37 ppm) and a broad one-proton singlet (1.64 ppm). The chemical shift of the six-proton singlet is indicative of two equivalent methyl groups attached to nitrogen. Addition of deuterium oxide to the ^1H NMR solution caused the disappearance of the resonance at 1.64 ppm. The infrared spectrum showed a carbonyl absorption (1685 cm^{-1}) and an absorption attributed to an acidic proton attached to nitrogen (3200 cm^{-1}). The odd molecular weight (m/e 157) as well as the ^1H NMR information demonstrated that three nitrogen atoms were present in the molecule. Based on this information the structure of 1,3-dimethyl-5-azabarbituric acid was proposed for compound 17 (see Figure 32). An authentic sample of compound 17 was prepared by allowing 2 equivalents of methylisocyanate to react with potassium cyanate and was shown to be identical in every respect with the caffeine ozonation product.[33]

Minor Products--

The identity of several caffeine ozonation products is based on GC/MS analysis since their yield was very low and their isolation in pure form was not effected. A compound 18 with molecular weight 226 was found in the product mixture (tr = 17.4, see Figure 30). This compound 18 gave a mass spectrum (see Figure 36) quite similar to that of 1,3-dimethyloxonic acid methylamide (compound 16, Figure 33). The molecular ion (m/e 226) fragmented by loss of 29 mass units and suggested the presence of a formyl group (CHO). Significant fragment ions at me/56, 83, and 141 mass units were formed in the

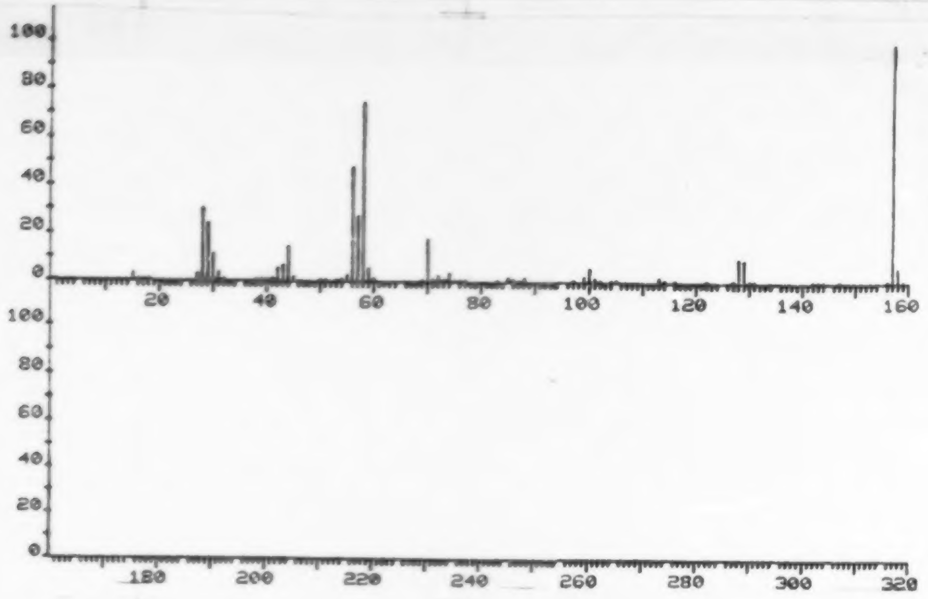

Figure 35. Mass spectrum of compound 17.

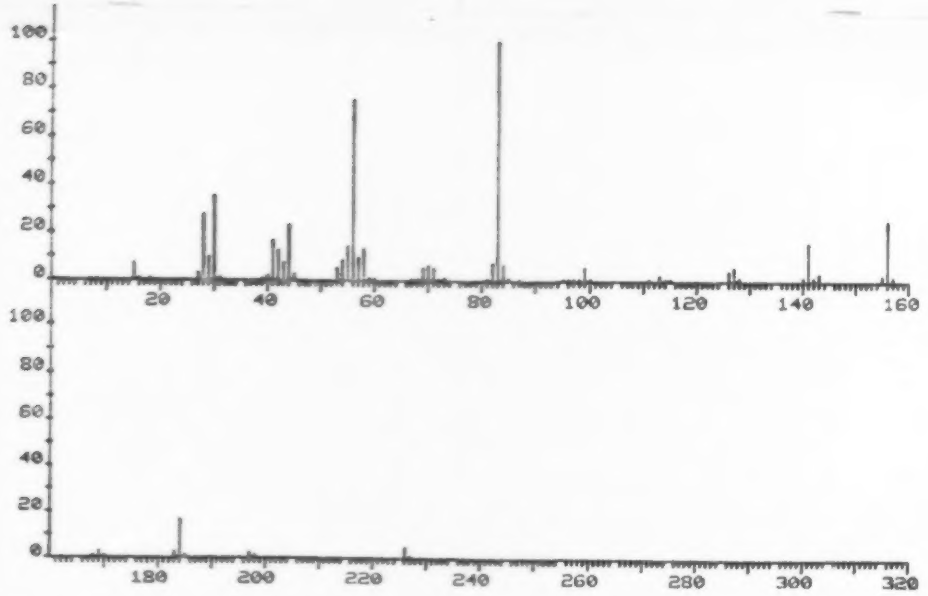

Figure 36. Mass spectrum of compound 18.

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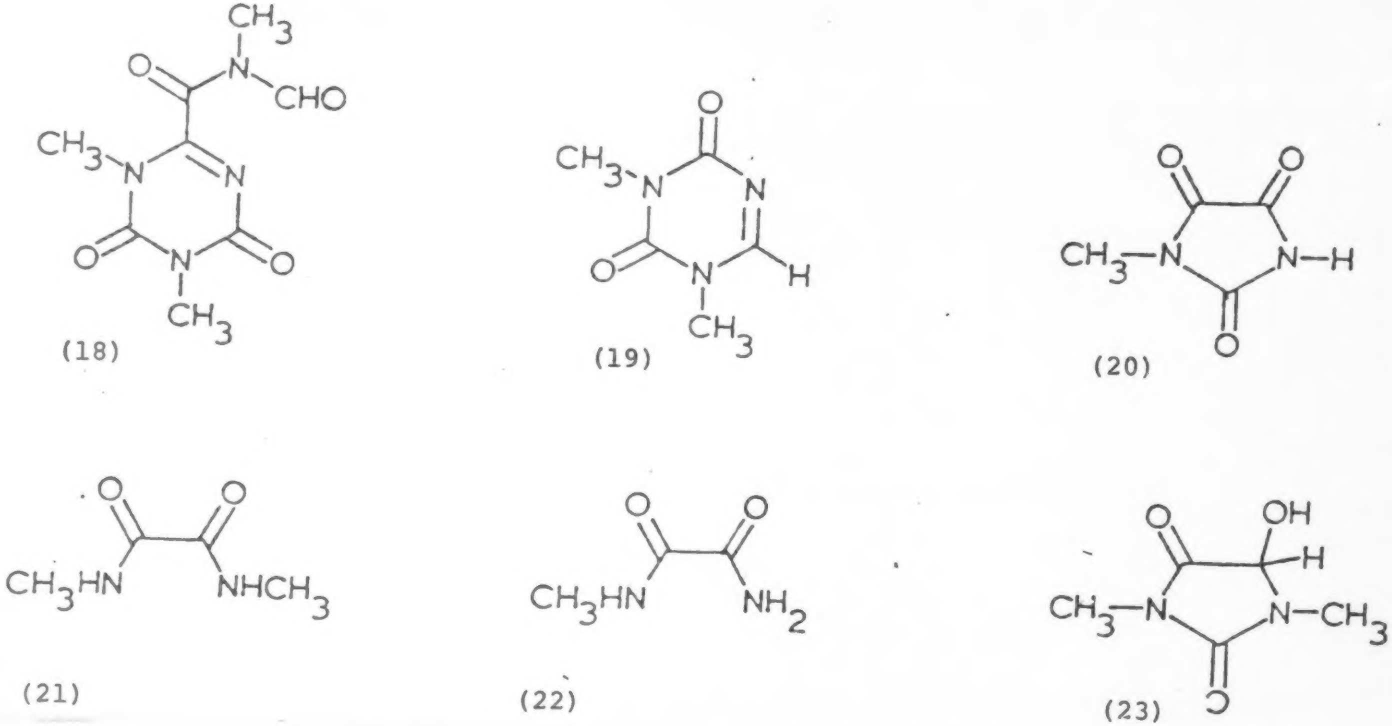
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Figure 37. Caffeine ozonation products.

mass spectrum of both compound 16 and 18. It was demonstrated through deuterium labeling experiments that methylisocyanate loss originated from carboxamide side chain and indicated that fragment ion 141 represented the triazine ring system. The similar nature of each mass spectrum below 141 mass units implies that an identical triazine ring system is present in both compounds 16 and 18. Based on this information structure 18 (see Figure 37) was proposed for this compound.

Compound 19 (t_r = 11.7 minutes) gave a mass spectrum with a molecular ion at 141 mass units (see Figure 38). The molecular ion fragments by loss of 28 and 57 mass units. In addition, significant fragment ions were formed at 42, 56, and 84 mass units. The odd molecular weight (m/e 141) suggested that compound 19 contained an odd number of nitrogen atoms, and a triazine ring was suspected since other products (16, 17, and 18) contained moiety. The structure (19) shown in Figure 37 was proposed for this compound. An authentic sample of 1,3-dimethyl-5-azauracil (18) was prepared by allowing 5-azauracil to react with sodium hydroxide and dimethyl sulfate and found to give a mass spectrum identical with the caffeine ozonation product. Gas chromatographic coinjection of the authentic sample with the product mixture supported the structural assignment for compound 19. Since, however, this ozonation product was not isolated, the identification is still considered tentative.

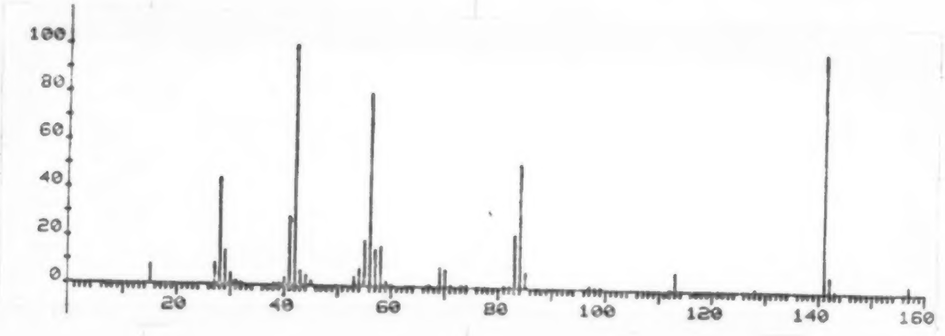

Figure 38. Mass spectrum of compound 19.

The mass spectrum of compound 29 (t_r = 12.3 min) showed a molecular ion at 128 mass units (see Figure 39). Ions at 141 and 157 units are molecular ions from compounds 17 (t_r = 10.5 min) and 19 (t_r = 11.7) which are tailing into the chromatographic peak of compound 20. The molecular ion of compound 20 (m/e 128) fragments by loss of 28 mass units. The even molecular weight suggests that an even number of nitrogen atoms are present. A parabanic acid derivative was suspected since this mass spectrum (Figure 39) showed similarities to that of dimethylparabanic acid (compound 2) by 14 mass units (a methylene unit). Based on this information, the structure shown for methylparabanic acid (20) was proposed (see Figure 37). An authentic sample of methylparabanic acid (20) was prepared by treating methylurea with oxalyl chloride and the product's mass spectrum found to be identical with the ozonation product. Gas chromatographic coinjection of the authentic sample with the product mixture supported the structural assignment of compound 20.

Compound 21 (t_r = 3.2 min) gave a mass spectrum that indicated a molecular ion at 116 mass units (see Figure 40). The molecular ion fragments by loss of 58 mass units to form the base peak in the spectrum (m/e 58). The even mass of the molecular ion indicated that the compound contained an even number of nitrogen atoms. The structure (21) shown in Figure 37 was proposed for this compound. An authentic sample of 1,4-dimethyloxalic acid amide was prepared by allowing methylamine to react with oxalychloride and the product's mass spectrum was identical to that of the ozonation product. A gas chromatography coinjection experiment of the authentic sample supported the proposed structural assignment.

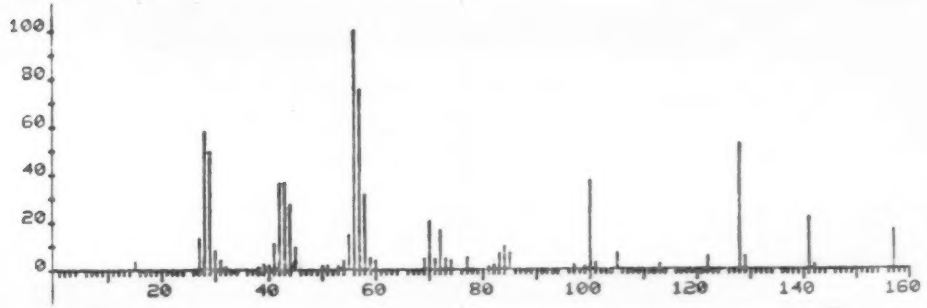

Figure 39. Mass spectrum of compound 20.

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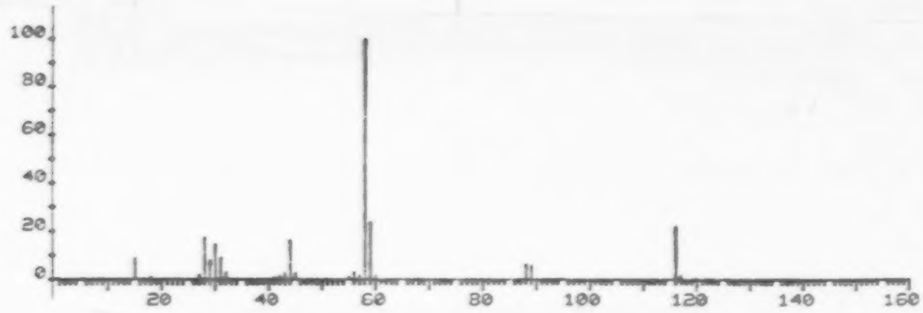


Figure 40. Mass spectrum of compound 21.

Compound 22 (t_r = 5.6 min) gave the mass spectrum shown in Figure 41 and indicated a molecular weight of 102 mass units. The ion at 142 mass units is a molecular ion from compound 2 (t_r = 4.5 min) which is tailing into the chromatographic peak of compound 22. The molecular ion fragments by loss of 58 and 44 mass units. This compound appears to be a derivative of oxalic acid amide since the mass spectrum is similar to that of 1,4-dimethyloxalic acid amide (21, see Figure 40) and differs in molecular weight by only 14 mass units. The mass spectrum of this compound is consistent with the one expected from 1-methyloxalic acid amide (see Figure 37).

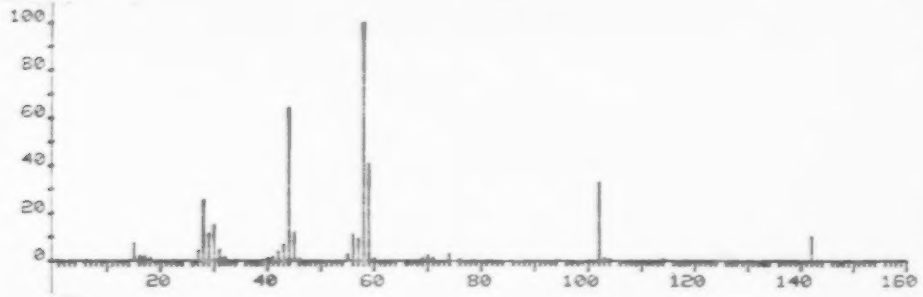


Figure 41. Mass spectrum of compound 22.

Compound 23 ($t_r = 10.1$ min) gave a molecular ion (m/e 144) which fragmented by loss of 28 mass units (see Figure 42). The mass spectrum of this minor caffeine ozonation product was found to be identical with that of the major ozonation product of dimethyluracil. This product was isolated from the dimethyluracil reaction mixture by column chromatography on silica gel and characterized by nuclear magnetic resonance, infrared, and mass spectrometry. A detailed discussion of this information is deferred until later in this section. Based on this information, the compound was assigned the structure (23) shown in Figure 37. An authentic sample of this material was not available for mass spectral comparison.

Ozonation of Other Xanthine Derivatives

The origin of specific atoms present in the caffeine ozonation products was determined by the use of ethyl and trideuteromethyl labels. 1-Ethyl-3,7-dimethylxanthine (24) was prepared by allowing an alkaline solution of 3,7-dimethylxanthine (25) to react with ethyl iodide. In a similar manner, 1-3, dimethyl-7-ethylxanthine (26) was prepared from 1,3-dimethylxanthine (27). The trideuteromethyl derivatives (compounds 28, 29, and 30) were prepared by allowing the appropriate dimethylxanthine to react with d_6-dimethyl sulfate under phase transfer alkylation conditions[17].

1,3-Dimethyl-7-Ethylxanthine

1,3-Dimethyl-7-ethylxanthine (26) was allowed to react with the ozone under caffeine ozonation conditions. The product mixture was analyzed by GC/MS techniques (see experimental section, conditions c) which revealed that products similar to those produced from caffeine (1), but some differing by 14 mass units, were produced. The mass spectral information indicated

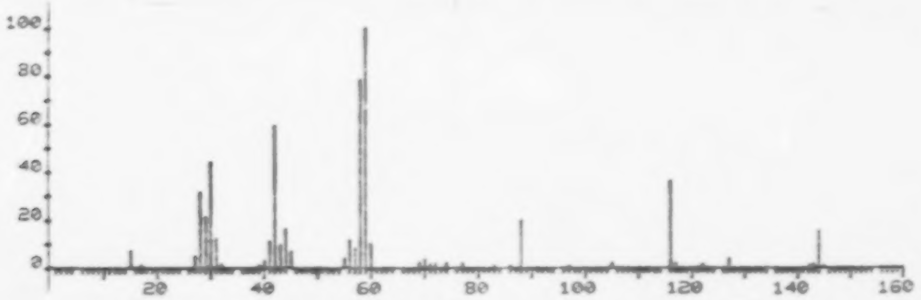
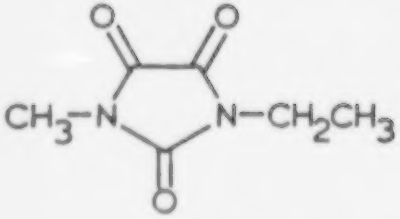


Figure 42. Mass spectrum of compound 23.



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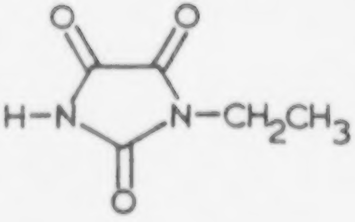

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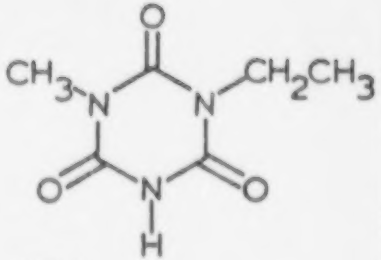

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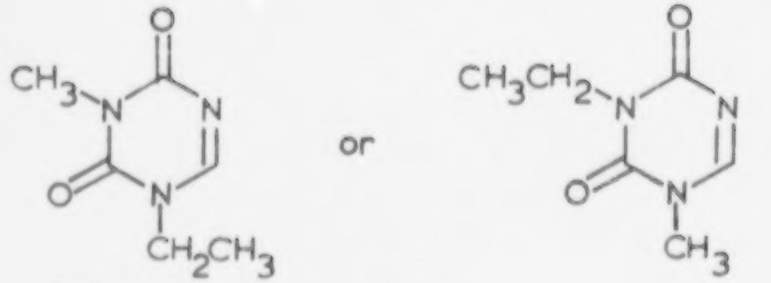

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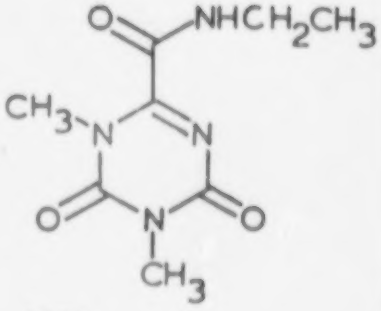


(35)

Figure 43. Ozonation products of 1,3-dimethyl-7-ethylxanthine.

that dimethylparabanic acid (2) was a major ozonation product (t_r = 5.4 min). Minor reaction products (31, 32, 33, and 34) are shown in Figure 43.

The compound (35) (t_r = 13.0 min) was produced during the ozonation reaction which gave a molecular ion at 212 mass units. The molecular ion fragmented by loss of 15, 28, and 71 mass units. The mass spectrum below 141 mass units closely resembled that of 1,3-dimethyloxonic acid methylamide (16) and implied that the same triazine ring system was present. Compound 35 was isolated from the ozonation reaction mixture by preparative column chromatography on silica gel. The ^{1}H NMR spectrum exhibited 5 resonances; 2 three-proton singlets (3.40 and 3.78 ppm), a three-proton triplet (1.26 ppm) a broad one-proton singlet (8.00 ppm). The infrared spectrum showed the presence of a carbonyl absorption (1660 cm^{-1}), a carbon-nitrogen double bond (1590 cm^{-1}), and an absorption (3350 cm^{-1}) attributed to a hydrogen atom attached to nitrogen. The ^{1}H NMR signal at 8.00 ppm disappeared upon addition of deuterium to the ^{1}H NMR solution and was attributed to an acidic hydrogen attached to nitrogen. The absence of a three-proton doublet, assigned to a methyl group attached to the carboxamide nitrogen in compound 16 demonstrated that the ethyl group must be attached to the carboxamide side chain in compound 35. Based on the above information, the structure of 1,3-dimethyloxonic acid ethyl amide (35) was assigned to this compound (see Figure 43; Figure 44 shows the mass spectrum).

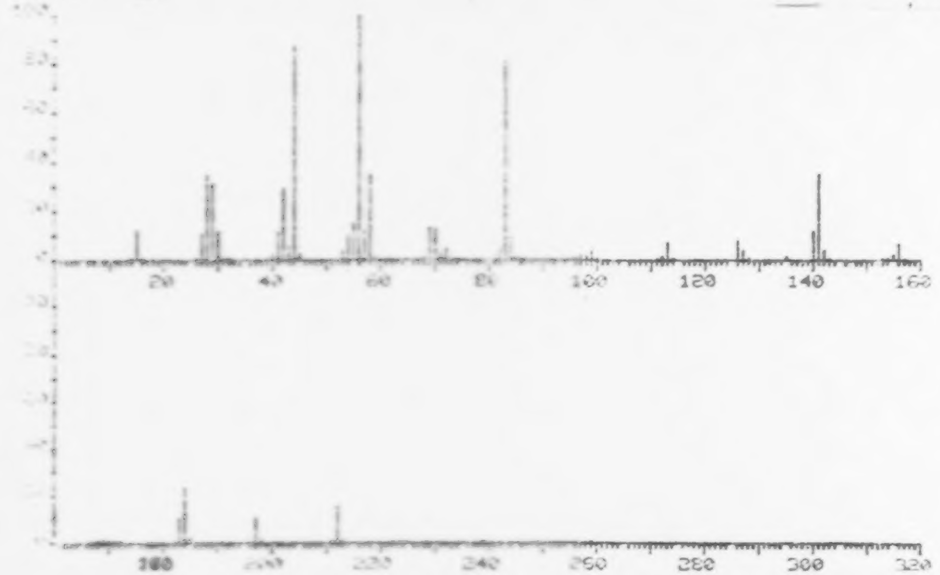
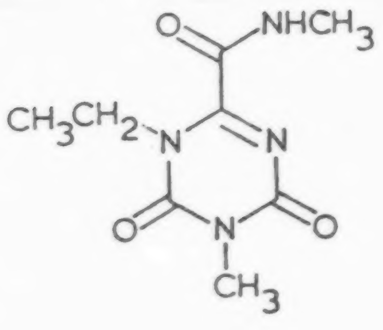


Figure 44. Mass spectrum of compound 35.

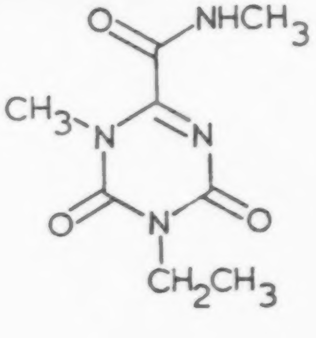
1-Ethyl-3,7-Dimethylxanthine

1-Ethyl-3,7-dimethylxanthine (24) was allowed to react with ozone under caffeine ozonation conditions. The product mixture was analyzed by GC/MS techniques (see experimental section, conditions C) which revealed that products similar to those produced from caffeine (1) and 1,3-dimethyl-7-ethylxanthine (24), but some differing by 14 mass units, were produced.

A new substance, compound 36 (t_r = 13.0 min), was isolated from the product mixture by prepartive column chromatography on silica gel. The mass spectrum indicated a molecular ion (m/e 212) which fragmented by loss of 15 and 57 mass units. The ^{1}H NMR spectrum showed 5 resonances, a three-proton doublet (3.00 ppm), a three proton singlet (3.44 ppm), a two-proton quartlet (4.44 ppm), and a broad one-proton singlet (7.76 ppm). Addition of deuterium oxide to the ^{1}H NMR solution caused the disappearance of the resonance at 7.76 ppm and the collapse of the doublet (3.00 ppm) to a singlet. The infrared spectrum showed the presence of a carbonyl group (1680 cm^{-1}), a carbon-nitrogen double bone (1600 cm^{-1}), and an absorption attributed to a hydrogen attached to nitrogen (3300 cm^{-1}). Two structures, A and B, were proposed for compound 36 (see Figure 45).



A



B

Figure 45. Proposed structures for compound 36.

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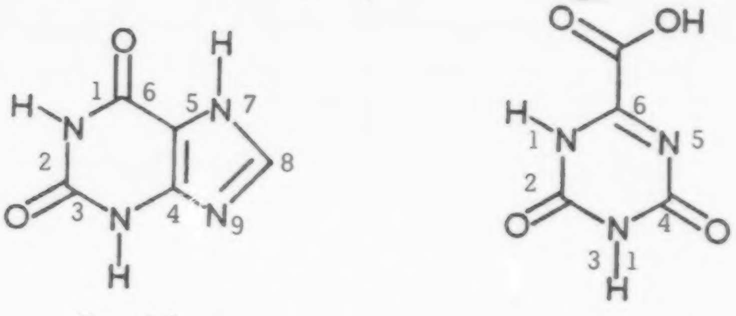
The identity of this compound as having either structure A or B is based on ^{1}H NMR chemical shift data. The methyl resonance at 3.40 ppm for compound 37 is consistent with that of a methyl group attached to an imide nitrogen (see Figure 46) and indicates that A is the correct structure (see Table 14).



Xanthine **Oxonic Acid**

Figure 46. Structure of xanthine and oxonic acid.

Mechanistic Implications

The ozonation of ethylated xanthines, compounds 24 and 27 provided mechanistic information concerning the formation of the major caffeine (1) ozonation product, 1,3-dimethyloxonic acid methylamide (16). The production of 1,3-dimethyl-7-ethylxanthine (26) demonstrated that the 7-position of the xanthine nucleus becomes the nitrogen atom of the carboxamide side originated from the 9-position of the xanthine nucleus since it contains no methyl group. The formation of 1-ethyl-3-methyloxonic acid methylamide (36) from 1-ethyl-3,7-dimethylxanthine indicated that the 1-, 2-, and 3- positions of the xanthine nucleus were incorporated into the triazine ring. The proposed origin of the remaining atoms of the oxonic acid derivatives is shown in figure 48 and assumes that minimal bond cleavage and recombination occur during product formation. In this scheme the 4-position of caffeine (1) becomes the remaining carbonyl carbon of the triazine ring. Apparently the 6-position becomes the imino carbon and the 5-position becomes the carbonyl carbon of the carboxamide side chain. Presumably carbon eight is lost as CO_2 during ozonation.

The results of these labeling experiments are rationalized in terms of the mechanism shown in Figure 49. It is proposed that ozonation of caffeine (1) occurs to yield the 9-membered ring structure D. The imino bond of D

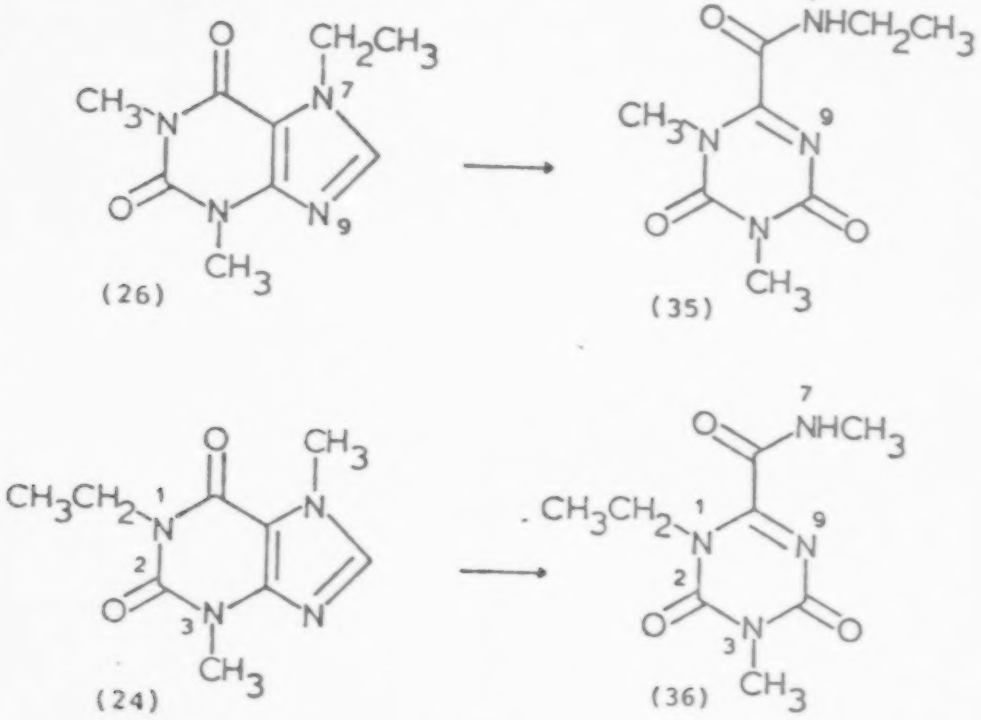


Figure 47. Results of ethyl labeling experiments.

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TABLE 14. PMR DATA ON XANTHINE AND OXONIC ACID DERIVATIVES[a]

	N_1 - Alkyl	N_3 - Alkyl	N_7 - Alkyl
1,3,7-Trimethylxanthine (1)	3.45 (s)	3.55 (s)	4.00 (s)
1,3-Dimethyloxonic acid methylamide (16)	3.75 (s)	3.40 (s)	3.00 (d)[c]
1-Ethyl-3,7-dimethylxanthine (24)	4.10 (q)[d]	3.58 (s)	4.00 (s)
1,3-Dimethyl-7-ethylxanthine (26)	3.43 (s)	3.59 (s)	4.45 (q)[c]
1-Ethyl-3-methyloxonic acid methylamide (37)	4.45 (q)[d]	3.40 (s)	3.00 (d)[c]
1,3-Dimethyloxonic acid ethylamide (35)	3.75 (s)	3.40 (s)	3.45 (m)[d]

[a] See Figure 46 for reference to parent compounds

[b] Multiplicity symbols are: s=singlet, d=doublet, q=quartet, m=multiplet

[c] The methyl signal is split into a doublet by the proton attached to nitrogen

[d] Only the methylene proton of the ethyl group is shown

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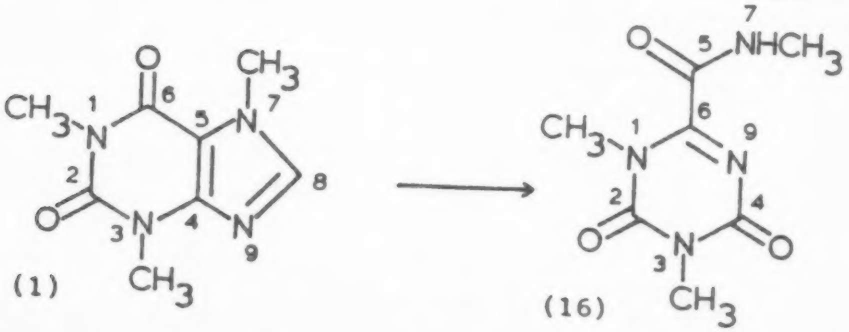
hydrolyzed to yield E. The 9-position (caffeine numbering) of E undergoes intramolecular nucleophilic addition to the carbonyl group (6-position) with subsequent loss of water to form the triazine ring system of F. The formyl group in F is oxidized by ozone to acid G which upon decarboxylation yields 1,3-dimethyl-oxonic acid methylamide (16).

Ozonation of Pyrimidine Derivatives

Trideuteriomethyl Caffeine Analogs--

The appearance of dimethylparabanic acid (2) and ethylmethylparabanic acid (31) in the ozonation products of 1-ethyl-3,7-dimethylxanthine (24) and 1,3-dimethyl-7-ethylxanthine (26) indicated that dimethylparabanic acid (2) is formed by at least two mechanistic pathways. Apparently dimethylparabanic acid (2) is formed in each case from combinations of the N-1 and N-7 positions. However, the latter possibility could not be determined from the above labeling experiments.

The relative amount of dimethylparabanic acid (2) formed from each possible combination was determined from the ozonation of caffeine analogs containing N-trideuteriomethyl labels. The aqueous ozonation of each analog (compounds 28, 29, and 30) produced dimethylparabanic acid (2) which contained both labeled (d_3) and unlabeled methyl groups. The integration of the molecular ion intensity of the unlabeled (m/e 142) and the labeled (m/e 145) materials gave the relative amount of each material contained in the product mixture. Since unlabeled dimethylparabanic acid (2) can be formed by only one combination of methyl groups in each experiment the relative percentage formed is the relative contribution from that pathway (see Table 15). Ozonation of 1-trideuteriomethyl-3,7-dimethylxanthine (28) produced 2 percent



(1) (16)

Figure 48. Origin of atoms in 1,3-dimethyl oxonic acid methylamide.

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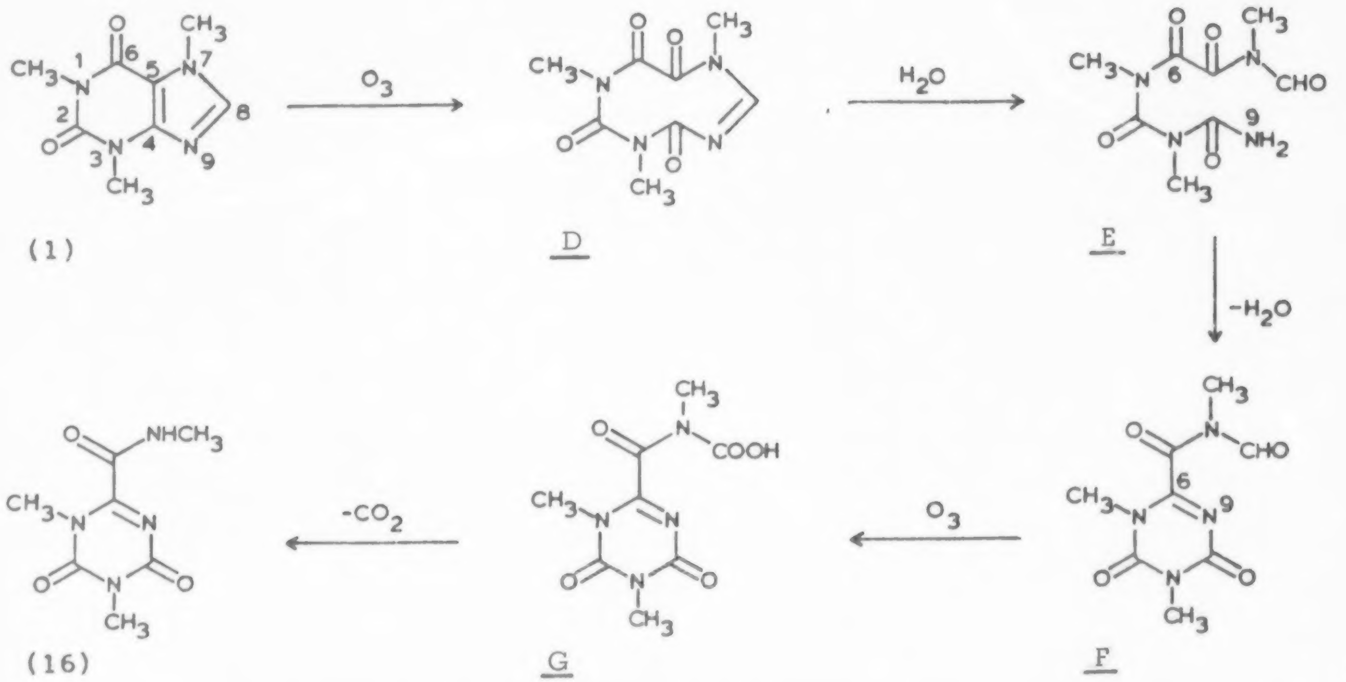
CH3
1
6
5
7
2
8
3
4
9
(1)
O3
D
H2O
E
6
9
NH2
CHO
-H2O
F
COOH
G
O3
-CO2
NHCH3
(16)

Figure 49. Proposed mechanism for the formation of 1,3-dimethyloxonic acid methylamide (16).

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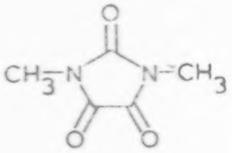
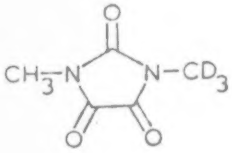
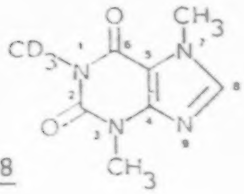
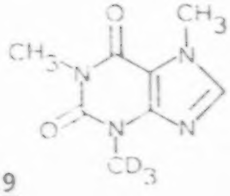
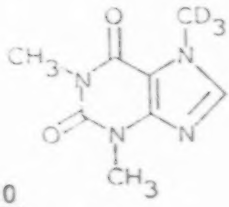
TABLE 15. DIMETHYLPARABANIC ACID FORMATION FROM DEUTERIUM LABELED CAFFEINE ANALOGS.

	Relative Percent	
Compound	 M$^+$ 142	 M$^+$ 145
 28	2	98
 29	12	88
 30	86	14

[a] Data collected by GC-MS experiment, conditions A.

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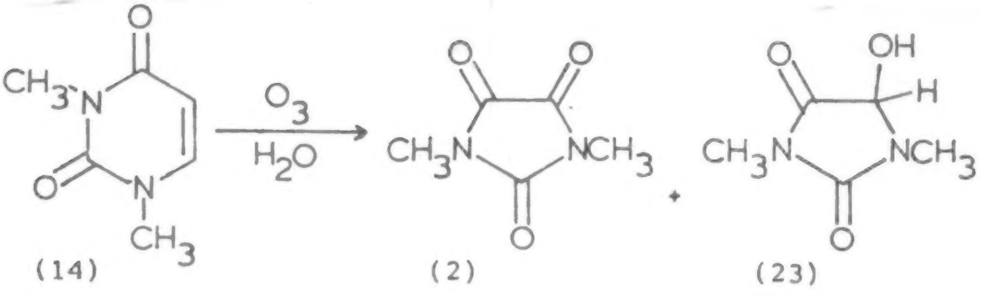
unlabeled dimethylparabanic acid and 98 percent labeled (d_3) dimethylparabanic acid. This demonstrated that 2 percent of the dimethylparabanic acid (2) was formed from combination of positions N-3 and N-7. Ozonation of 1,7-dimethyl-3-trideuteriomethylxanthine (29) produced 12 percent unlabeled dimethylparabanic acid and 88 percent labeled (d_3) dimethylparabanic acid. Ozonation of 1,3-dimethyl-7-trideuteriomethylxanthine (30) produced 86 percent unlabeled dimethylparabanic acid and 14 percent labeled (d_3) dimethylparabanic acid. These results demonstrate that 12 percent dimethylparabanic acid (2) was formed by combination of N-1 and N-7 positions, 86 percent formed from the N-1 and N-3 positions, and 2 percent from the N-3 and N-7 positions.

1,3-Dimethyluracil--

Since most of the dimethylparabanic acid (2) originated from the pyrimidine ring system (86%) of caffeine, the ozonation of the corresponding monocyclic compound, 1,3-dimethyluracil (14), was investigated to see if similiarities exist (see Figure 50). 1,3-dimethyluracil (14) was prepared by allowing uracil to react with an alkaline solution of dimethylsulfate.[35] The aqueous ozonation of 1,3-dimethyluracil (14) produced two major products, dimethylparabanic acid (2) and 1,3-dimethyl-5-hydroxyhydantoin (23.) Compound 23 was isolated from the reaction mixture by column chromatography and shown by mass spectrometry to have molecular weight 144. The spectrum was found to be identical with that obtained on a minor caffeine ozonation product (see Figure 39). The ^{1}H NMR spectrum showed three resonances



(14) (2) (23)

Figure 50. Ozonation of 1,3-dimethyluracil (14).

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a six-proton singlet (3.02 ppm), a one-proton singlet (5.20 ppm), and one proton singlet (5.50 ppm). Addition of deuterium oxide to the ^{1}H NMR solution caused the singlet at 5.50 ppm to disappear. The IR spectrum showed the presence of an alcohol absorption (3350 cm^{-1}) and a carbonyl absorption (1710 cm^{-1}). When compound 23 was allowed to react with ozone under 1,3-dimethyluracil (14) ozonation conditions it was recovered unchanged. When the ozonation was conducted in the presence of $FeSO_4$, compound 23 was completely converted to dimethylparabanic acid (2). Based on this information, compound 23 was assigned the structure of 1,3-dimethyl-5-hydroxyhydantoin (see Figure 51). The above ozonation experiments also demonstrate that compound 23 is not a precusor of dimethylparabanic acid (2).

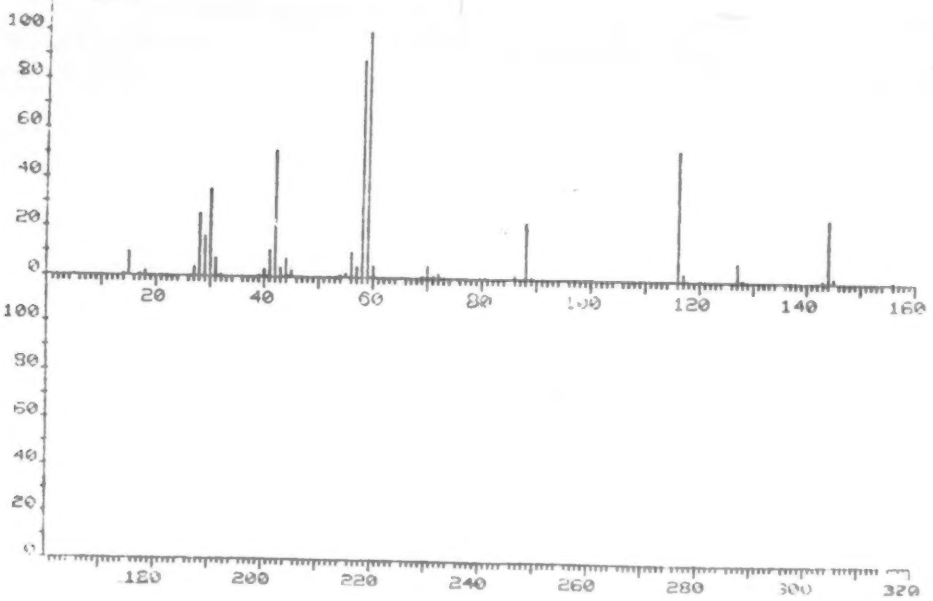
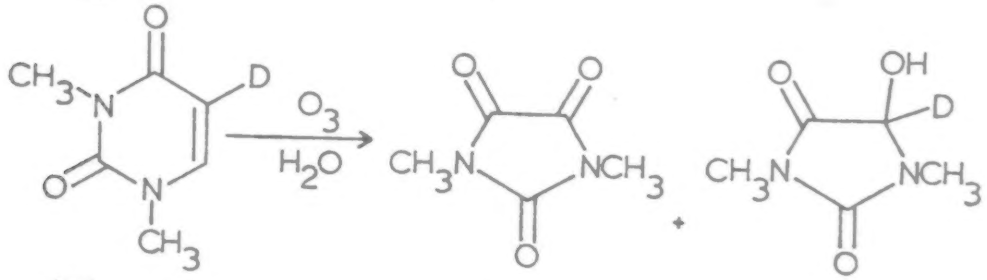


Figure 51. Mass spectrum of 1,3-dimethyl-5-hydroxyhydantoin (23) formation.

The formation of dimethylparabanic acid (2) from 1,3-dimethyluracil (14) occurs with loss of a carbon atom (see Figure 50) presumably from either the 5- or 6-position of compound 14. The loss of either position 5 or 6 upon ozonation could be determined by the incorporation of an isotopic carbon label at either position 5 or 6 in compound 14. The preparation of either labeled material with a carbon-13 or carbon-14 label would require an expensive and lengthy process and was not attempted for this reason.

The formation of 1,3-dimethyl-5-hydroxyhydantoin (23) also occurs with loss of either the 5 or 6 carbon of 1,3-dimethyluracil (14). However, in this case the product (compound 23) retains a hydrogen atom at the 5 position of compound 23. This hydrogen atom must originate from either the 5 or 6 position of 1,3-dimethyluracil (14). Ozonation of a 5 or 6 deuteriouracil derivative would reveal which carbon is lost upon formation of 1,3-dimethyl-5-hydroxyhydantoin (23). 1,3-dimethyl-5-deuteriouracil (37) was prepared in good yield and high isotopic purity (89 percent, 99 percent d_1) by acid catalyzed exchange of 1,3-dimethyluracil (14) in deuterium oxide.

The ozonation of 1,3-dimethyl-5-deuteriouracil (43) produced dimethylparabanic acid (2) and 1,3-dimethyl-5-hydroxy-5-deuteriouracil (38) as determined by GC/MS analysis (see Figure 52). A mass spectrum of compound 38 demonstrated that isotopic purity is identical (99 percent d_1) to that of the starting material, compound 37. This result demonstrates that the 5-position of 1,3-dimethyluracil (14) is retained in the product and becomes the 5-position of 1,3-dimethyl-5-hydroxyhydantoin (23). The 6-position of 1,3-dimethyluracil (14) is apparently lost as carbon dioxide.



(37) (2) (38)

Figure 52. Ozonation of 1,3-dimethyl-5-deuteriouracil (37).

The above results can be rationalized by the mechanism shown in Figure 53. Ozonation of 1,3-dimethyluracil (14) occurs to given dialdehyde

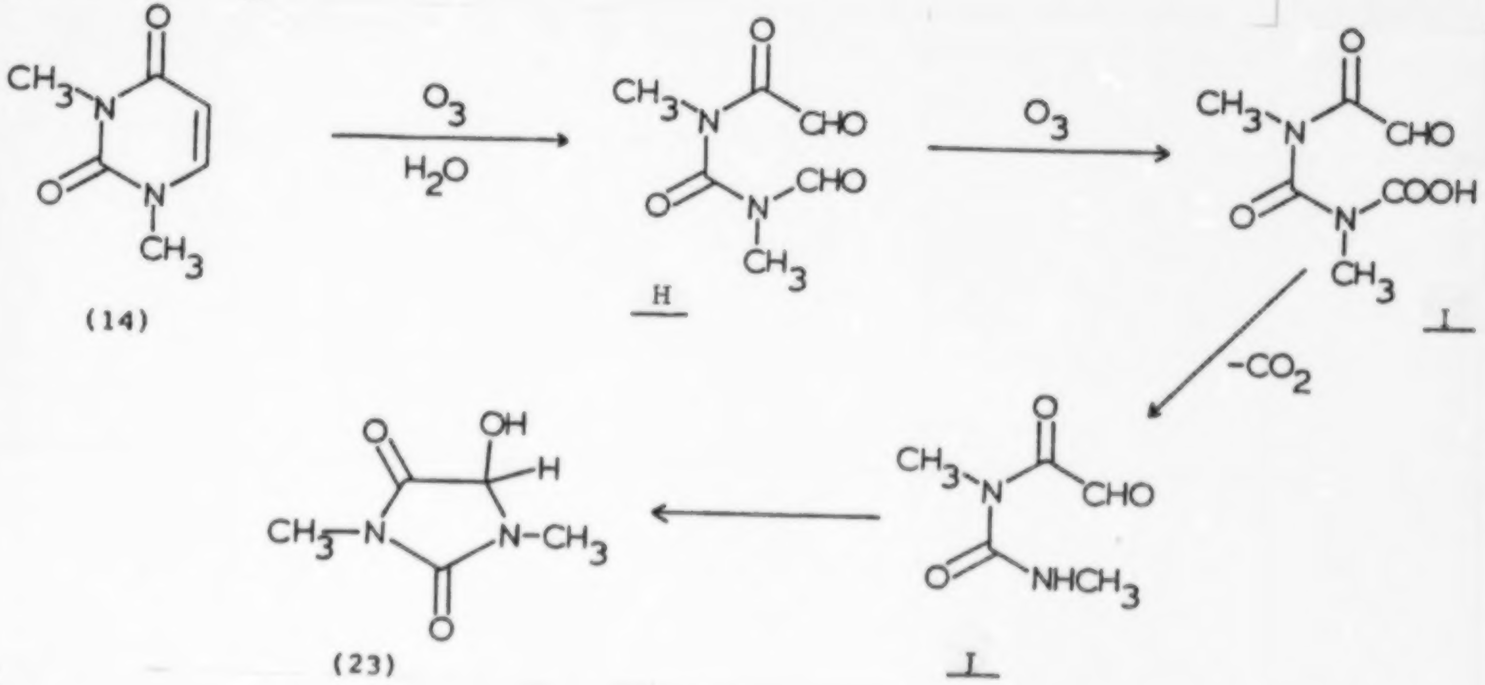

O
CH3-N
O
N
CH3
(14)
O3
H2O
CH3-N
O
CHO
O
N
CHO
CH3
H
O3
CH3-N
O
CHO
O
N
COOH
CH3
I
-CO2
CH3-N
O
CHO
O
NHCH3
I
O
OH
H
CH3-N
N-CH3
O
(23)

Figure 53. Proposed mechanism for 1,3-dimethyl-5-hydroxyhydantoin (23) formation.

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H. The dialdehyde (H) undergoes further oxidation to acid I which upon decarboxylation yields J. Intermediate J cyclizes to form 1,3-dimethyl-5-hydroxyhydantoin (23).

1,3,5-Trimethyluracil--

The ozonation of 1,3,5-trimethyluracil (15) proceeds in a similar manner to that of 1,3-dimethyluracil (14). The oxonation reaction of compound 15 yields two major products neither of which are dimethylparabanic acid (2). Apparently substitution of a methyl group at the five position of 1,3-dimethyluracil (14) prevents dimethylparabanic acid (2) formation.

The first product (compound 39) gave a mass spectrum (conditions C, Temp 1, 100°C; Temp 2, 250°C; Rate = 8°C/min) which showed a molecular ion at m/e 101. The molecular ion fragments by loss of 28, 43, and 58 mass units. Compound 39 was isolated from the product mixture by column chromatography. The ^{1}H NMR spectrum indicated 3 resonances; a three-proton singlet (2.48 ppm), a three-proton doublet (2.86 ppm), and a one-proton singlet (3.14 ppm). The resonance at 2.86 ppm indicated the presence of a N-methylcarboxamide function in the molecule. Based on this information the structure of pyruvic acid N-methylamide (39) was proposed for this compound (see Figure 54).

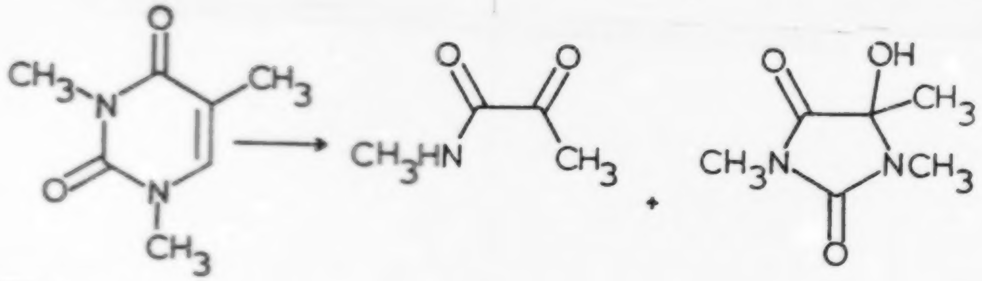
(15) (39) (40)

Figure 54. Ozonation of 1,3,5-trimethyluracil (15).

The second compound (40) showed a molecular ion (m/e 158) which fragments by loss of 15, 17, and 28 mass units. The molecular weight of compound 46 differed from that of 1,3-dimethyl-5-hydroxyhydantoin (23) by 14 mass units. In addition, the mass spectral fragmentation patterns of both compounds were quite similar. Based on this information and mechanistic information concerning the ozonation of 1,3-dimethyl-uracil (14), the structure of 1,3,5-trimethyl-5-hydroxyhydantoin (40) was tentatively proposed for this compound (see Figure 54).

Mechanistic Implications: Dimethylparabanic Acid Formation--

There are several possible mechanisms for the formation of dimethylparabanic acid (2). One possible mechanism is shown in Figure 55. In this scheme, 1,3-dimethyluracil (14) is ozonated to dialdehyde H. In this case the five position (uracil numbering) is oxidized to hydrotrioxide K. A hydrotrioxide intermediate has been proposed by Murray for the ozonation of an aldehyde to an acid.[36] Intermediate K undergoes further oxidation with loss of carbon dioxide to form amide L. Intermediate L cyclizes to form tetrahedral intermediate M. Intermediate M loses hydroxide and singlet oxygen to produce dimethylparabanic acid (2). Alternatively, hydrotrioxide intermediate K could decompose to acid N which then undergoes conversion by a similar scheme to dimethylparabanic acid (2). Attempts to gain support for this proposed mechanism have not been successful.

Another possible mechanism for dimethylparabanic acid formation is shown in Figure 56. In this scheme ozonation of 1,3-dimethyluracil (14) occurs to give ozonide O. Alternatively an epoxide (P) might be formed. Either ozonide O or epoxide P undergo rearrangement to form aldehyde Q. Aldehyde intermediate Q undergoes tautomerization to enol from R which produces dimethylparabanic acid (2) upon ozonation. Precedent for the rearrangement of acyl substituted epoxide is found in the conversion of chalcones, alpha-beta unsaturated carbonyl compounds to alpha-formyldeoxybenzoins.[37]

Attempts to gain support for this mechanism were not successful. The attempted preparation of aldehyde intermediate Q by allowing the lithium enolate of 1,3-dimethylydantoin to react with ethylformate was not successful. However, the product mixture from the ozonation of 1,3-dimethyluracil (14) does contain a minor component of molecular weight (m/e 156) which would correspond to that of epoxide P or aldehyde Q. This component was not present in sufficient abundance to allow collection of other meaningful mass spectral data.

Conclusion

The ozonation of caffeine (1) yields several major products and many minor products. These results demonstrate that the ozonation reaction of xanthine and pyrimidine derivatives occurs by diverse mechanistic paths. 1,3-dimethyloxonic acid methylamide (16) is formed upon caffeine (1) ozonation by one distinct mechanistic pathway. Dimethylparabanic acid (2), however, is formed upon caffeine (1) ozonation by at least three distinct mechanistic pathways. If the ozonation of caffeine proceeds by type of mechanism

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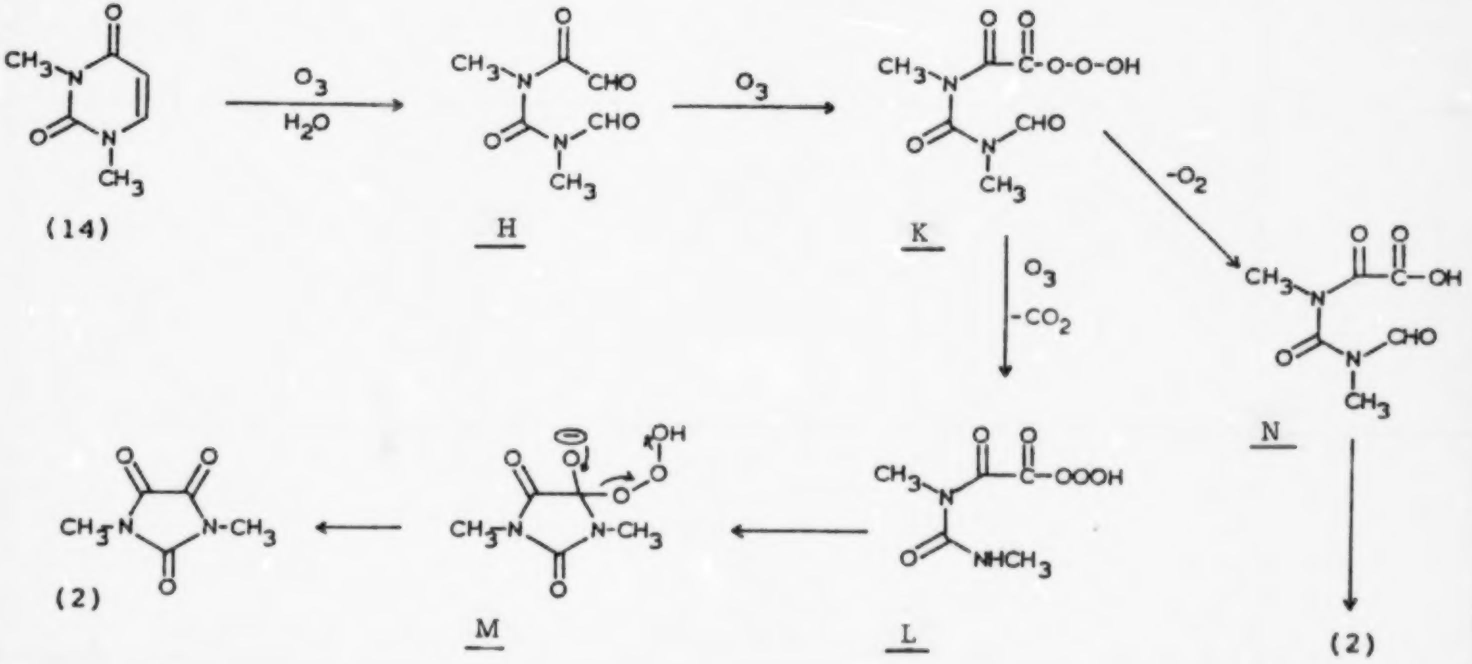

Figure 55. Proposed mechanism (A) for formation of dimethylparabanic acid (2).

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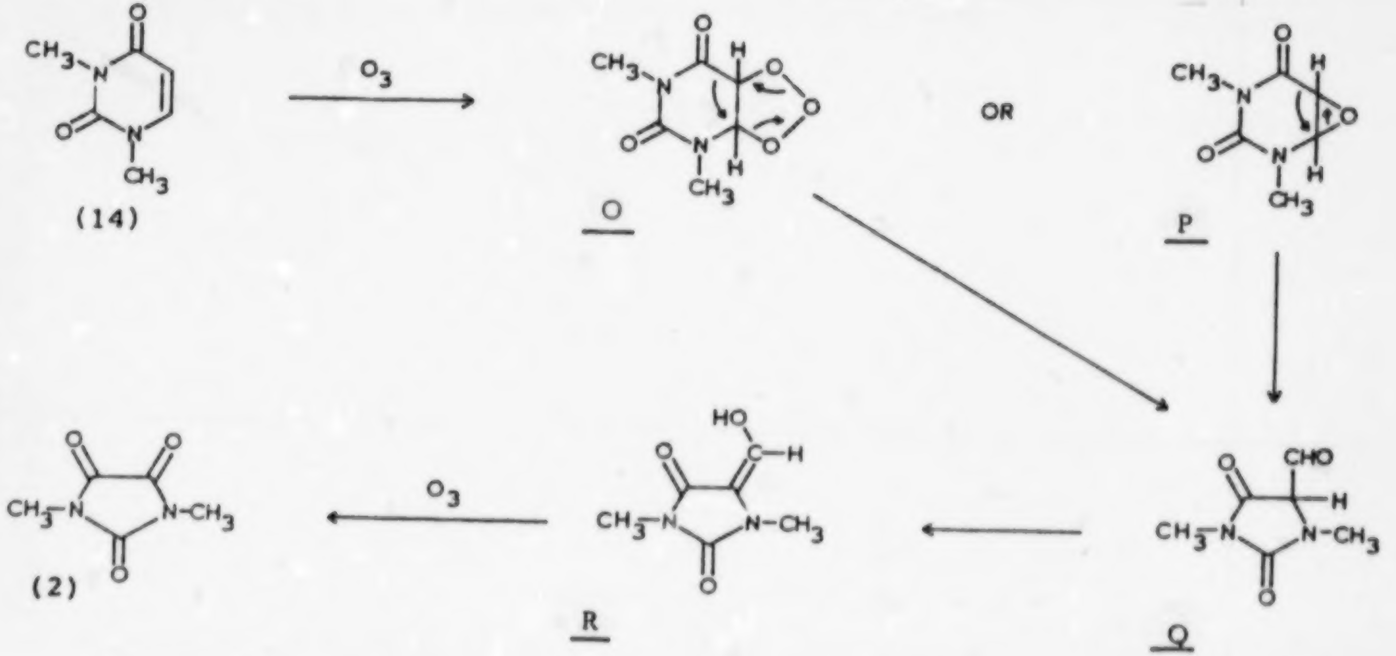

Figure 56. Proposed mechanism (B) for formation of dimethylparabanic acid (2).

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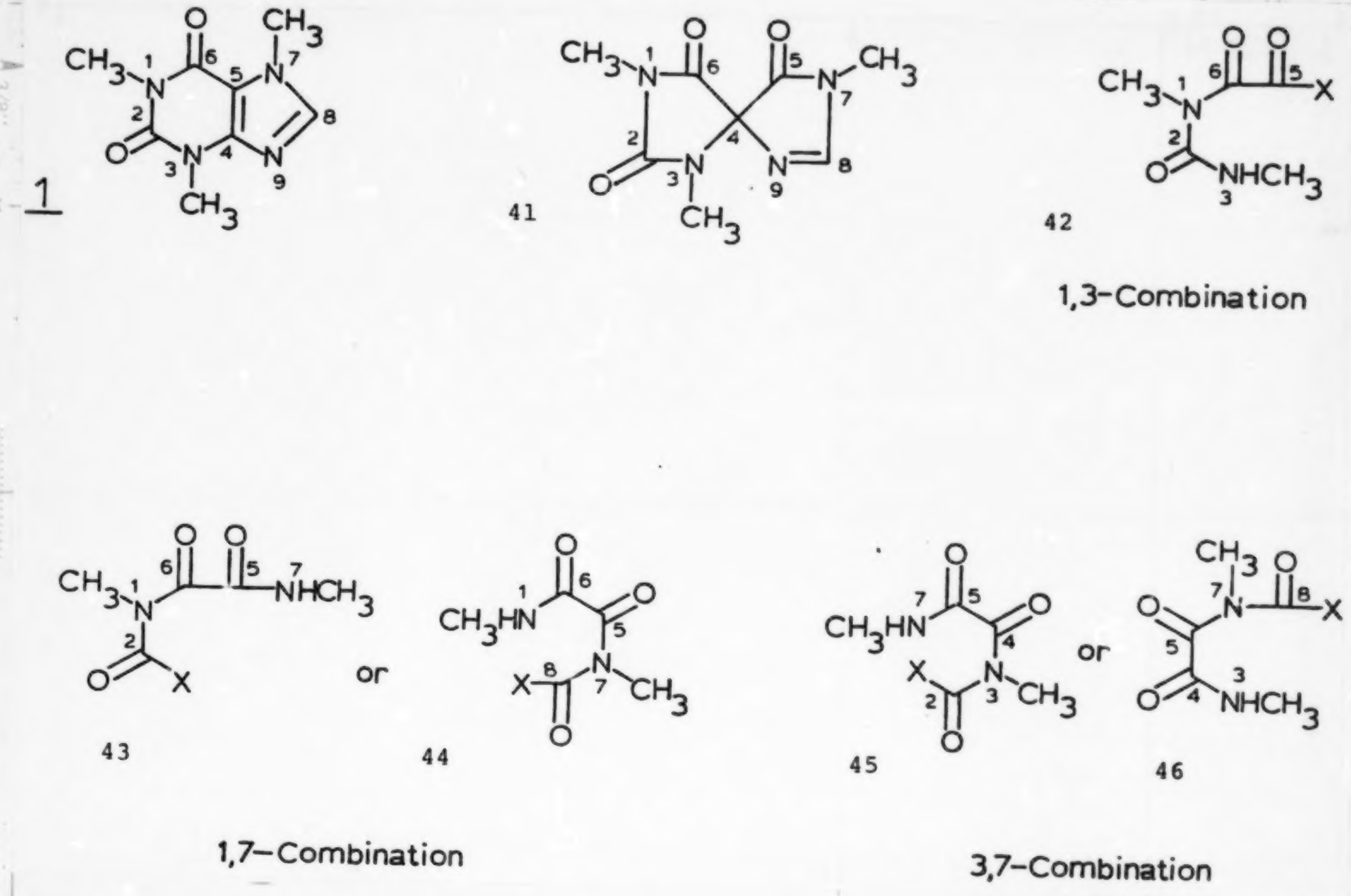
1
41
42
1,3-Combination
43
or
44
45
or
46
1,7–Combination
3,7–Combination

Figure 57. Formation of dimethylparabanic acid from caffeine.

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shown in Figure 56 for 1,3-dimethyluracil (14), then the intermediate (41) shown below would be formed. Biltz proposed that similar spiro compound are oxidative reaction products of substituted uric acids.[38] Ozone induced decomposition of intermediate 41 to dimethylparabanic acid (2) would explain cnly one mechanism for dimethylparabanic acid (2) formation. All three mechanistic pathways can be explained if intermediates of the type shown in Figure 57 (42-46) are formed upon caffeine (1) ozonation. Intermediates of this type might result from ozone induced decomposition of intermediates D or E shown in Figure 49. In Figure 57, X represents a suitable leaving group; a hydroxl, hydrotrioxide, or peroxide linkage are possible leaving groups. The results of this study indicates that Figure 55 shows the more likely mechanism for dimethylparabanic acid (2) formation. However, neither mechanism shown in Figure 55 and Figure 56 or a combination of each, can be excluded from these results.

Experimental Section

Organic free distilled water was obtained by distillation from a $KMnO_4$-KOH (50:50) solution. Methyl isocyanate, 5-azauracil, dimethylsulfate, oxalyl chloride, ethylurea, 1,3-dimethylurea, ethyliodide, and diazald were obtained from the Aldrich Chemical Company, Inc. Caffeine and methylurea were obtained from Eastman Kodak Company. Theobromine, theophylline, thymine, and uracil were obtained from the Sigma Chemical Company. Potassium Cyanate was obtained from Matheson, Coleman and Bell. Dimethyl-d_6 sulfate was obtained from Merck Sharp and Dohme Canada Limited. 1,3-Dimethyl-4-methylcarbamoylimino-1,3-imidazoline-2,5-dione was prepared by the procedure of Patton (see reference 18). Chlorine gas was obtained from the Matheson Gas Company. Silica Gel Woelm (0.031-0.063 mm) for column chromatography was obtained from ICN Pharmaceuticals Inc. Precoated thin layer chromatography plates (silica gel, thickness; 0.25 mm) were obtained from Van Waters and Rogers Scientific.

Liquid chromatography was conducted on a 30 mm x 1000 mm Altec glass Column packed with silica gel Woelm and equipped with a 15 mm x 150 mm precolumn (silica gel). Solvents were delivered at a 100 psi by a fluid metering pump (FMI, model RSPY). Typical flow rates range from 10-19 mL/min. Gas chromatography-mass spectrometry (GCMS) was conducted on a Hewlett-Packard 5982 A GC-MS computer system. A membrane separator was employed to interface the gas chromatograph to the mass spectrometer. Proton magnetic resonance (^1H NMR) spectra were obtained on a Varian EM 390 MHz spectrometer. Carbon magnetic resonance (^{13}C NMR) spectra were obtained on a JEOL PFT-100 spectrometer equipped with a Nicolet Model 1080 data system. Infrared spectra were obtained on a Perkin Elmer 337 Grating Infrared Spectrometer. Melting points were obtained on a Thomas Hoover capillary melting point apparatus and are corrected. Gas chromatography was conducted on a Hewlett-Packard model 5830 gas chromatograph equipped with

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glass columns and a FID detector. Gas chromatography conditions are as follows: Conditions A; Column, Aue pack[39] (2mm X 10 ft), 100-120 mesh; Temp 1, 100°C; Time 1, 0 min; Rate = 8°C/min; Temp 2, 240°C; Time 2, 10 min; Inj Temp, 250°C; Flow (N_2), 30 mL/min; Mass scan 10-300 mass units. Conditions B; Column, Aue pack (2 mm X 10 ft), 100-120 mesh; Temp 1, 100° C; Time 1, 0 min; Rate - 10°C/min; Temp 2, 240°C; Time 2, 30 min; Inj temp, 240°C; FID Temp, 250; Chart speed, 0.5 inch/min. Conditions C; Column, DC-710 (5%, 2 mm X 10 ft), on Chromasorb Q, 80-100 mesh; Temp 1, 150°C; Time 1, 4 min; Rate = 16°C/min; Temp 2, 220°C; Time 2, 20 min; Inj temp, 250°C; Flow (N_2), 30 mL/min; Mass scan, 10-300 mass units. Conditions D; Column, Aue pack (2 mm x 6 ft), 100-120 mesh, Conditions B, except Slope sensitivity, 1:00. It was determined that the Aue pack columns (conditions A, B, and D) were best suited for chromatography of xanthine derivatives and their ozonation products.

The following data established that caffeine was the compound at a retention time of 22.0 min in Figure 25. Mass spectrum (70 ev) m/e (rel intensity) 80(15), 193(27), 109(45), 82(51), 67(60), 194, M+(100). This structural assignment was verified by the coinjection of an authentic sample of caffeine with the original sample. Caffeine (authentic sample), mass spectrum (70 ev) m/e (rel intensity) 82(16), 193(16), 55(27), 67(27), 109(44), 194, M+ (100).

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Ozonation of Caffeine (I)--

Caffeine (0.5 g, 2.6 mmol) was dissolved in pure distilled water (750 mL) and placed in a 1-liter 3-neck flask equipped with magnetic stirrer, gas dispersion tube, and a bubble flow meter. The solution was allowed to react for 90 min with an ozone-oxygen stream (600 mL/min, 17 mg O_3/min). The resulting solution was sparged with nitrogen (600 mL/min) for 20 min, transferred to a round bottom flask, and concentrated by roto-evaporation. The residue was dissolved in 50 mL of $CH_3OH:CH_2Cl_2$ (50:50) and dried over anhydrous Na_2SO_4. This mixture was filtered and concentrated by rotoevaporation. The residue was transferred to a vial and the volume adjusted to 1.0 mL by addition of CH_3OH. This sample was analyzed by gas chromatography-mass spectrometry to yield the data shown below. Gas chromatography was conducted to determine the relative product distribution (results are not corrected for variance in detector response). Coinjection experiments were conducted according to conditions D. The data are presented as: Compound, retention time (conditions A), (Rel percent, conditions B); Mass spectrum (70 ev) m/e (rel intensity). 1,4-Dimethyloxalamide, 3.2 min, (0.6%); MS, 88(8), 59(20), 44(32), 116, M+(33), 30(44), 59(100). Coinjection, ozonation product, t_r = 3.80 min, (coinjection with authentic sample, t_r = 3.87 min). Dimethylparabanic acid, 4.5 min, (23.0%); MS 48(5), 114(10), 70(19), 57(48), 56(60), 58(80), 142, M+(100). Coinjection, ozonation product, t_r = 4.98 min, (coinjection with authentic sample, t_r = 5.09 min). 1-Methyloxalamide, 5.6 min, (1.6%); MS 45(10), 102, M+(10), 59(30), 44(34), 30(45), 32(56), 31(65), 58(100). 1,3-Dimethyl-5-hydroxyhydantoin, 10.1

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(2.7%); MS 127(7), 56(18), 88(22), 144, M+(23), 116(51), 42(70), 58(87), 59 (100). Coinjection, ozonation product, $t_r = 9.81$ min, (coinjection with authentic sample, $t_r = 10.01$ min). 1,3-Dimethyl-5-azabarbituric acid, 10.5, min, (8.7%); MS 129(8), 100(9), 44(19), 70(22), 57(31), 56(51), 58(74), 157, M+ (100). Coinjection, ozonation product, $t_r = 10.07$ min, (coinjection with authentic sample, $t_r = 10.13$ min). 1,3-Dimethyl-5-azauracil, 11.7 min, (3.4%); MS 83(12), 29(12), 41(18), 84(30), 28(37), 42(98), 141, M+(100). Coinjection, ozonation product, $t_r = 10.93$ min, (coinjection with authentic sample, $t_r = 10.94$ min). Methylparabanic acid, 12.3 min, (5.8%); MS 42 (16), 44(22), 70(24), 43(34), 100(41), 57(61), 128, M+(70), 56(100). Coinjection, ozonation product, $t_r = 11.53$ min, (coinjection with authentic sample, $t_r = 11.57$ min). 1,3-Dimethyloxonic acid methylamide, 14.9 min, (26.4%); MS 113(7), 198, M+(31), 141(32), 170(38), 58(42), 44(44), 83(80), 56(100).

Determination of Ozone-Caffeine Stochiometry--

A 1-liter 3-neck flask (reaction vessel) containing pure water (750 mL) was placed in line with a 2-liter three neck flask containing 1.5 liters of 2% KI solution. Ozone/oxygen (600 mL/min) was passed through the system for 1.5 hr. The 1.5 liter solution of $KI-I_2$ was transferred to a 2-liter volumetric flask and diluted to volume with water. Three 100 mL aliquots were removed and titrated with standard $Na_2S_2O_3$ solution (0.1031 N) to give the following results: Titer, 31.84, 31.94, and 31.94 mL. The amount of ozone produced within a 1.5 hr. period was determined to be 1.58 g (17.7 mg O_3/min). A second experiment was conducted under the above conditions with caffeine (0.5000 g, 2.57 mmol) present in the reaction vessel. The amount of unreacted ozone which passed into the KI trap in a 1.5 hr. period was determined to be 1.07 g O_3 (KI titer, 21.52, 21.60, and 21.56 mL; Ave = 21.56 mL). The amount of O_3 which reacted with caffeine was found to be 0.52 g or 10.2 mmol ozone. The number of moles ozone reacted per mole caffeine reacted was 4.2 moles ozone per mole caffeine.

Isolation of Dimethylparabanic Acid (2)--

A quantity of caffeine (1.0 g, 5.2 mmol) in water (750 mL) was ozonized as described above (ozonation time, 3 hr.). The solution was sparged with N_2 (600 mL/min) and extracted 3 times with $CHCl_3$ (200 mL). The $CHCl_3$ layers were combined, dried over Na_2SO_4, and filtered. The resulting filtrate was concentrated by roto-evaporation. The remaining residue was analyzed by gas chromatography (conditions A) and found to contain one component (retention time, 6.9 min). This residue crystallized upon cooling and was vacuum dried. The crystalline material was recrystallized from 95% ethanol, collected by suction filtration, and vacuum dried to yield 0.2 g (27%) dimethylparabanic acid (2): mp 153°C; (KBr pellet) 3200, 1735, 1460, 1385, 1280, 1100, 760 cm^{-1}. ^1H NMR ($CDCl_3$) delta 3.20 (s). ^{13}C NMR ($CDCl_3$) proton decoupled relative to TMS 24.9, 154.1, 157.0 ppm. Mass

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spectrum (70 ev) m/e (rel intensity) 114 (10), 29(18), 70(18), 57(30), 28(40), 56(47), 58(54), 142, M+(100).

Isolation of 1,3-Dimethyloxonic Acid Methylamide (3)--

A sample of caffeine (5.0 g, 25.8 mmol) in water (750 mL) was allowed to react with an ozone-oxygen stream (600 mL/min, 17 mg O_3/min) for 50 min. The mixture was sparged with N_2 (600 mL/min) for 20 min and concentrated by roto-evaporation. This residue was transferred to a vial and the volume adjusted to 10 mL with CH_2Cl_2:CH_3OH 50:50). Thin layer chromatography (TLC) of this sample on silica gel with THF:EtOAc (50:50) showed 3 major products: 1,3-Dimethylparabanic acid (2), ($R_f = 0.63$); 1,3-Dimethyloxonic acid methylamide (3), ($R_f = 0.40$); Caffeine (1), ($R_f = 0.20$). This sample was subjected to column chromatography through silica gel with THF:EtOAc (50:50). Fractions were collected at 10 mL intervals and analyzed by TLC. The fractions containing 1,3-dimethyloxonic acid methylamide (3) were combined and concentrated. This concentrated sample was again chromatographed through silica gel to yield fractions containing pure product. These fractions were combined and concentrated by roto-evaporation to yield an oil which crystallized upon scratching. This material was vacuum dried to yield 65 mg of 1,3-dimethyloxonic acid methylamide (3): mp 178-179°C; IR (KBr Pellet) 3160, 2930, 1740, 1670, 1595, 1475, 1435, 1425, 1385, 1360, 1275, 1180, 1020, 810, 785, 760, 680 cm^{-1}. ^1H NMR ($CDCl_3$) delta 2.98 (d,3), 3.42 (s,3), 3.78 (s,3), 7.80 (s, broad, 1). The addition of D_2O to the NMR sample caused the disappearance of the resonance at 7.80 ppm and the collapse of the doublet at 2.98 ppm. ^{13}C NMR ($CDCl_3$) proton decoupled spectrum, ppm relative to TMS, 26.5, 29.3, 33.5, 151.5, 154.5, 156.1, 159.7 ppm. Mass spectrum (70 ev) m/e (rel intensity) 29(11), 41(16), 42(16), 30(19), 15(21), 198, M+ (30), 141(33), 170(37), 28(40), 58(42), 44(46), 83(81), 56(100).

Isolation of 1,3-Dimethyl-5-azabarbituric Acid (4)--

A sample of caffeine (20.0 g, 103 mmol) in water (1500 mL) was allowed to react overnight with an ozone-oxygen stream (600 mL/min, 17 mg O_3/min). The resulting solution was sparged with N_2 (600 mL/min) for 20 min and extracted 4 times with CH_2Cl_2 (250 mL) to remove dimethylparabanic acid from the aqueous phase. The aqueous phase was concentrated to an oil. This oil was dissolved in EtOH:CH_2Cl_2 (200 ml, 50:50) and passed through a short column of anhydrous Na_2SO_4. This dry organic phase was concentrated to 30 mL. A 15 mL aliquot of this sample was subjected to column chromatography through silica gel with $CHCl_3$:THF:EtOAc (60:20:20). Chromatographic fractions were collected at 20 mL intervals and allowed to evaporate to dryness. The crystalline materials which were isolated in fractions 21-40 were combined and recrystallized (EtOH) to yield 100 mg 1,3-dimethyl-5-azabarbituric acid: mp 220-221 °C; IR (KBr pellet) 3200, 3050, 1685, 1460, 1385, 1280, 1100, 1050, 965, 760, 680 cm^{-1}. ^1H NMR ($CDCl_3$) delta 3.30 (s). ^{13}C NMR ($CDCl_3$) proton decoupled spectrum, ppm relative to TMS, 24.9, 154.1, 157.0 ppm. Mass spectrum (70 ev) m/e (rel intensity) 114(11), 29 (14), 70(14), 28(30), 57(30), 56(39), 58(51), 142, M+(100).

Preparation of 1,3-Dimethyl-5-azabarbituric Acid (4)--

A mixture of potassium isocyanate (2.0 g, 24.6 mmol) and methylisocyanate (5.7 g, 100 mmol) in DMF (100 mL) was allowed to react for 24 hr. at 75°. The DMF was removed by roto-evaporation to yield a solid residue. The residue was slurried in water (50 mL) and filtered to remove 1,3,5-trimethyl-5-azabarbituric acid. The filtrate was acidified with conc HCl and filtered to yield 2.2 g or crude 1,3-dimethyl-5-azabarbituric acid (57%). Mp 221-222°C IR (KBr pellet) 3290, 3050, 1660, 1450, 1390, 1075, 1010, 840, 760, 650 cm^{-1}. ^{1}H NMR (CDCl$_3$) delta 3.34 (s,6), 1.62 (s,1). Mass spectrum (70 ev) m/e (rel intensity) 128(11), 29(16), 57(16), 70(18), 28(28), 56 (35), 58(53), 157, M+ (100).

Preparation of N-methylparabanic acid (5)

A mixture of methylurea (4.5 g, 60.8 mmol) and oxalyl chloride (7.5 g, 59.1 mmol) in Et$_2$O was refluxed for two hrs. The Et$_2$O was removed by roto-evaporation to yield a crystalline solid. The solid was recrystallized to yield 5.3 g of N-methylparabanic acid (70%) Mp 155-157°C IR (KBr) (pellet). 3190, 1710, 1450, 1385, 1320, 1130, 1030, 770, 725, 690, 665 cm^{-1}. ^{1}H NMR (CDCl$_3$) delta 3.26 (s,3), 1.70 (s,1). Mass spectrum (70 ev) m/e (rel intensity) 41(11), 45(11), 55(14), 27(16), 42(16), 58(18), 72(19), 70(28), 43 (46), 29(51), 100(51), 28(72), 44(72), 128, M+(75), 57(79), 56(100).

Preparation of 1,3-Dimethyl-5-azauracil (8)--

A quantity of 5-azauracil (0.2 g, 1.77 mmol) was dissolved in a solution of NaOH (0.2 g, 5.0 mmol) in water (5 mL). Dimethyl sulfate (0.5 mL, 5.3 mmol) was added and the solution brought to a gentle boil. The solution was allowed to cool to room temperature and extracted 3 times with CH$_2$Cl$_2$ (5 mL). The CH$_2$Cl$_2$ extracts were combined and passed through a short column of anhydrous Na$_2$SO$_4$. The CH$_2$Cl$_2$ was removed by rotoevaporation to yield 0.1 g of 1,3-dimethyl-5-azauracil (40%). Analytically pure material was obtained upon recrystallization from EtOH. mp 155-157°C.

IR(KBr): 3000, 1666, 1590, 1455, 1430, 1370, 1345, 1235, 1135, 1020, 950, 830, 790, 770 cm^{-1}.

PMR(CDCl$_3$,): 3.40(s,3), 3.45(s,3), 8.11(s,1) ppm.

MS(m/e(rel. int.)): 58(10), 56(17), 41(21), 84(28), 28(30), 42(98), 141M+ , 100).

Ozonation of Caffeine (1) in Methanol--

A quantity of caffeine (0.1 g, 5.2 mmol) was slurried in absolute

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methanol (100 mL) and allowed to react with an ozone-oxygen stream (600 mL/min, 17 mg O_3/min) for three hours. The solution was sparged with N_2 (600 mL/min, 20 min) and concentrated by roto-evaporation. The solution was analyzed by GC and allowed to stand in a vial at room temperature. After several months, a large single crystal formed and was removed. GC-MS established that minor amounts of normal, aqueous caffeine ozonation products were present in the mother liquid. The crystal was vacuum dried to yield 0.1 g 1,3-dimethyloxonic acid N-methyl-N-dimethoxyformamide. mp 137-139°C. IR (KBr pellet) 2960, 1720, 1670, 1500, 1450, 1420, 1390, 1340, 1200, 1100, 1060, 920, 750 cm^{-1}. ^{1}H NMR ($CDCl_3$) delta 3.00 (s,3) 3.26 (s,6), 3.32 (s,3), 3.64 (s,3), 6.60 (s,1). Mass spectrum (70 ev) m/e (rel intensity) 72(13), 43(18), 56(15), 42(31), 58(35), 73(36), 172(100), 241, M+-31, (7).

Preparation of 1-Ethyl-3,7-dimethylxanthine (12)--

A quantity of theobromine (10.0 g, 55.6 mmol) was slurried in EtOH (50 mL) and a solution of KOH (5.0 g, 89.2 mmol) in EtoH (50 mL) was added. Upon cooling to 40°C a precipitate formed. A minimum amount of water (30 mL) was added to dissolve the precipitate. A solution of ethyl iodide (15.8 g, 101 mmol) in EtOH (20 mL) was added and the solution refluxed for 6 hrs. The solution was concentrated to 50 mL by roto-evaporation and 50 mL of water was added. The mixture was extracted 3 times with CH_2Cl_2 (100 mL). The CH_2Cl_2 layers were combined, dried over anhydrous Na_2SO_4, filtered, and evaporated by roto-evaporation. The resulting solid was sublimed to yield 9.0 g of 1-ethyl-3,7-dimethylxanthine (78%): mp 163-165°C. IR (KBr pellet) 3080, 2950, 1690, 1640, 1540, 1480, 1440, 1350, 1240, 1030, 890, 765, 615 cm^{-1}; ^{1}H NMR ($CDCl_3$) delta (s,1). ^{13}C NMR ($CDCl_3$ proton decoupled spectrum, ppm relative to TMS, 13.3, 29.6, 33.5, 36.5, 107.7, 141.3, 148.7, 151.3, 148.7, 151.3, 155.1 ppm. Mass spectrum (70 ev) m/e (rel intensity) 137(11), 165(11), 209(11), 70(12), 82(16), 179(16), 193 (16), 42(21), 55(23), 67(34), 109(30), 180(48), 208, M+(100).

Preparation of 1,3-Dimethyl-7-ethylxanthine (13)--

Following the above procedure for preparation of 1-ethyl-3,7-dimethylxanthine, theophylline (10.0 g, 55.6 mmol), KOH (5.0 g, 89.2 mmol) in EtOH (50 mL), and ethyl iodide (15.8 g, 101 mmol) yielded 9.5 g of 1,3-dimethyl-7-ethylxanthine (82%). mp 148-150°C. IR (KBr pellet) 3050, 1690, 1640, 1540, 1400, 1220, 1020, 975, 765, 750, 680 cm^{-1}. ^{1}H NMR ($CDCl_3$) delta 1.56 (t,3), 3.40 (s,3), 3.68 (s,3), 4.40 (q,2), 7.66 (s,1). ^{13}C NMR ($CDCl_3$) proton decoupled spectrum, ppm relative to TMS, 16.6, 27.9, 29.7, 42.3, 106.8, 140.3, 148.9, 151.6, 154.9. Mass spectrum (70 ev) m/e (rel intensity) 137(11), 165(11), 209(11), 70(12), 82(16), 179(16), 193(16), 42(21), 55(23), 67(34), 109(30), 180(48), 208, M+ (100).

Preparation of 1,3-Dimethyl-7-ethylxanthine (13)--

Following the above procedure for preparation of 1-ethyl-3,7-dimethylxanthine, theophylline (10.0 g, 55.6 mmol), KOH (5.0 g, 89.2 mmol) in EtOH

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(50 mL), and ethyl iodide (15.8 g, 101 mmol) yielded 9.5 g of 1,3-dimethyl-7-ethylxanthine (82%). mp 148-150°C. IR (KRb pellet) 3050, 1690, 1640, 1540, 1220, 1020, 975, 765, 680 cm^{-1}. ^{1}H NMR ($CDCl_3$) delta 1.56 (t,3), 3.40 (s,2), 3.68 (s,3), 4.40 (q,2), 7.66 (s,1). ^{13}C NMR ($CDCl_3$) proton decoupled spectrum, ppm relative to TMS, 16.6, 27.9, 29.7, 42.3, 106.8, 140.3, 148.9, 151.6, 154.9. Mass spectrum (70 ev) m/e (rel intensity) 29 (11), 42(11), 94(11), 209(11), 96(12), 123(14), 180(20), 67(32), 193(37), 95 (82), 208, M+(100).

Ozonation of 1-Ethyl-3,7-dimethylxanthine (12)--

Following the above procedure for the ozonation of caffeine, a quantity of 1-ethyl-3,7-dimethylxanthine (0.5 g, 2.4 mmol) in water (750 mL) was allowed to react with ozone-oxygen. The product mixture was subjected to gas chromatographic-mass spectral analysis (conditions C) to yield the following mass spectral data. Compound, retention time, mass spectrum (70 ev) m/e (rel intensity). 1,4-dimethyloxalamide, 1,8 min, 74(11), 57(12), 41(16), 31 (18), 116, M+(32), 58(33), 30(81), 43(100). Dimethylparabanic acid, 2.5 min, 70(26), 56(46), 56(77), 58(89), 142(100). Ethylmethylparabanic acid, 2.8 min, 72(11), 42(12), 57(14), 57(14), 27(16), 29(18), 70(28), 43(30), 28 (55), 58(35), 156, M+(86), 56(100). 1-Ethyl-3-methyl-5-hydroxhydantoin (tentative identification), 4.6 min, 130(11), 143(11), 27(12), 42(16), 56(16), 69(16), 102(18), 158, M+(18), 73(26), 29(28), 44(28), 30(30), 28(46), 58 (100). N-Methylparabanic acid and N-Ethylparabanic acid, 5.1 min, 100(12), 42(16), 27(18), 44(23), 71(23), 128, M+ (N-methylparabanic acid, 23), 57 (25), 70(25), 58(26), 29(32), 142, M+(N-ethylparabanic acid, 32), 43(37), 28 (60), 56(100). 1-Ethyl-3-methyl-5-azabarbituric acid, 5.8 min, 99(11), 57 (12), 100(12), 42(16), 113(16), 29(19), 43(25), 58(26), 144(33), 70(37), 28 (46), 156(39), 171, M+(84), 56(100). 1-Ethyl-3-methyloxonic acid methylamide, 10.9 min, 42(11), 70(11), 97(11), 27(12), 56(14), 29(16), 55(21), 28 (26), 128(32), 212, M+(32), 69(39), 58(100).

Ozonation of 1,3-Dimethyl-7-ethylxanthine (13)--

Following the above procedure for the ozonation of caffeine, a quantity of 1,3-dimethyl-7-ethylxanthine (0.5 g, 2.4 mmol) in water (750 mL) was allowed to react with ozone-oxygen and the product mixture subjected to GC-MS analysis (conditions C) to yield the following mass spectral data. Compound, retention time, mass spectrum (70 ev) m/e (rel intensity). Dimethylparabanic acid, 5.4 min, 114(11), 70(18), 29(26), 57(40), 28(40), 56(54), 58(77), 142, M+(100). Ethylparabanic acid, 5.6 min, 71(14), 27(18), 43(21), 57(21), 70 (30), 29(32), 58(42), 28(58), 156, M+(95), 56(100). Methylparabanic acid and ethylparabanic acid, 7.4 min, 59(11), 58(12), 100(12), 27(16), 44(18), 42(19), 57(21), 29(23), 128, M+ (methylparabanic acid, 23), 70(26), 71(32), 28(39), 43(40), 142, M+ (ethylparabanic acid, 47), 56(100). 1,3-Dimethyl-5-azabarbituric acid, 8.1 min, 44(11), 128(11), 70(18), 29(21), 57(21), 28 (30), 56(40), 58(51), 157, M+(100). 1,3-Dimethyl-5-azauracil, 9.9 min, 57 (12), 29(16), 83(16), 41(21), 56(35), 28(39), 84(39), 42(88), 141, M+(100).

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1,3-Dimethyloxonic acid ethylamide, 13.0 min, 41(11), 57(11), 140(11), 197 (11), 27(12), 30(12), 69(12), 70(12), 55(16), 212, M+(16), 184(21), 42(30), 29(32), 28(35), 58(35), 141(55), 83(81), 44(88), 56(100).

Isolation of Ethylmethylparabanic Acid (14)--

A quantity of 1-ethyl-3,7-dimethylxanthine (5.0 g, 24 mmol) in water (750 mL) was allowed to react for 12 hrs. with an ozone-oxygen stream (600 mL/min, 17 mg O_3/min). The solution was sparged with N_2 (600 mL/min) for 20 min and extracted three times with CH_2Cl_2(200 mL). The CH_2Cl_2 extracts were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated by roto-evaporation. The resulting oil was subjected to bulb to bulb distillation at reduced pressure to yield 1.1 g ethylmethylparabanic acid (29%). mp 42-44°C. IR (KBr pellet) 2970, 1720, 1450, 1410, 1390, 1350, 1265, 1220, 1110, 1070, 820, 760 cm^{-1}. ^{1}H NMR ($CDCl_3$) delta 1.30 (t,3), 3.20 (s,3), 3.72 (q,2). Mass spectrum (70 ev) m/e (rel intensity) 72(11), 42(12), 57(14), 27 (16), 29(18), 70(28), 43(30), 28(35), 58(35), 156, M+(86), 56(100).

Preparation of Ethylparabanic Acid (14)--

Following the procedure for preparation of methylparabanic acid, ethylurea (10.0 g, 114 mmol) and oxalyl chloride (14.4 g, 114 mmol) yielded 9.9 of ethylparabanic acid (61%). mp 91-94°C. IR (KBr pellet) 3200, 1700, 1450, 1420, 1375, 1340, 1300, 1125, 1070, 920, 770, 715, 660 cm^{-1}. ^{1}H NMR ($CDCl_3$) delta 1.30 (t,3), 1.86 (s, broad, 1), 3.70 (q,2). Mass spectrum (70 ev) m/e (rel intensity) 29(11), 27(12), 42(12), 44(14), 28(18), 70(28), 43 (37), 71(44), 142, M+(68), 56(100).

Preparation of Ethylmethylparabanic Acid (14)--

A quantity of ethylparabanic acid (5.0 g, 35 mmol) in CH_2Cl_2 (250 mL) was allowed to react (3.5 hr, room temperature) with excess CH_2N_2 (70 mmol). The excess CH_2N_2 was destroyed with HOAc and the solvent removed by roto-evaporation to yield an oil. The oil was distilled at reduced pressure to yield 4.2 g of ethylmethylparabanic acid (76%). mp 42-44°C. IR (KBr pellet) 2950, 1710, 1450, 1400, 1375, 1360, 1265, 1210, 1120, 1090, 1060, 910, 760 cm^{-1}. ^{1}H NRM ($CDCl_3$) delta 1.30 (t,3), 3.20 (s,3), 3.75 (q,2). Mass spectrum (70 ev) m/e (rel intensity) 44(11), 27(14), 42(14), 57(16), 29(18), 70(30), 58(32), 29(33), 43(33), 156, M+(77), 56(100).

Isolation of 1-Ethyl-3-Methyloxonic Acid methylamide (17)--

A quantity of 1-ethyl-3,7-dimethylxanthine (10.0 g, 48 mmol) in water (750 mL) was allowed to react for 12 hrs with an ozone-oxygen stream (600 mL/min, 17 mg O_3/min). The solution was sparged with N_2 (600 mL/min) and extracted three times with CH_2Cl_2 (200 mL). The aqueous phase was concentrated to an oil by roto-evaporation. The oil was dissolved in 100 mL of CH_2Cl_2:CH_3OH (50:50) and passed through a column of anhydrous Na_2SO_4. This solution was concentrated to 20 mL and chromatographed through silica gel with $CHCl_3$:CH_3OH (19:1). Fractions of the effluent (20 mL) were

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collected and allowed to evaporate. The composition of these fractions was determined by GC analysis. The contents of fractions 45, 46, and 47 (which contained the desired product) were combined, evaporated, and the residue recrystallized from EtOH to yield 0.2 of 1-ethyl-3-methyloxonic acid methylamide. mp 182-184°C. IR (KBr pellet) 3300, 2900, 1740, 1660, 1600, 1460, 1360, 1160, 1030, 800 cm^{-1}) delta 1.40 (t,3), 3.00 (d,3), 3.44 (s,3), 4.44 (q,2), 7.76 (s,broad, 1). ^{13}C NMR (CDCl$_3$) proton decoupled spectrum, ppm relative to TMS, 14.7, 26.5, 29.3, 42.5, 150.7, 154.3, 155.7, 159.5. Mass spectrum, (70 ev) m/z (rel intensity) 70(11), 155(11), 29(12), 97(12), 140(12), 56(14), 28(18), 55(18), 212, M+(35), 69(40), 128(44), 58(100).

Isolation of 1,3-Dimethyloxonic acid ethylamide (15)--

A quantity of 1,3-dimethyl-7-ethylxanthine (7.5 g, 35 mmol) and FeSO$_4$ (5.4 g, 35 mmol) was dissolved in water (500 mL0 and allowed to react four hrs with an ozone-oxygen stream (600 mL/min, 17 mg O$_3$/min). The solution was sparged with N$_2$ (600 mL/min, 20 min) and celite was added. The slurry was filtered to remove an iron precipitate and the filtrate was concentrated by roto-evaporation. The residue was shaken in the presence of several boiling stones and 100 mL of CH$_2$Cl$_2$:CH$_3$OH (50:50). Small amount of celite was added and the solution was filtered. The filtrate was concentrated to m0 mL and chromatographed through silica gel with CHCl$_3$:CH$_3$OH (19:1). Solvent fractions were collected (10 mL) and analyzed by GC. Fractions 120-160 were combined and evaporated to yield 271 mg of 1,3-dimethyloxonic acid ethylamide: mp 145-147°C. IR (KBr pellet) 3350, 2900, 1740, 1660, 1590, 1430, 1150, 840, 780, 760, 675 cm^{-1}. ^{1}H NMR(CDCl$_3$) delta 1.26 (t,3), 3.40 (s,3), 3.50 (q,2), 3.78 (s,3), 8.00 (s, broad, 1). Mass spectrum (70 ev) m/z (rel intensity) 57(11), 126(11), 55(12), 140(12), 212, M+ (12), 70 (14), 183(14), 69(16), 184(16), 28(23), 29(25), 42(25), 141(33), 58(35), 83 (65), 56(86), 44(100).

Preparation of 1-Trideuteromethyl-3,7-dimethylxanthine (21)--

A quantity of theobromine (1.0 g, 5.4 mmol), triethylbenzylammonium chloride (0.4 g, 1.8 mmol), sodium hydroxide (0.5 g, 12.5 mmol) were partitioned between chloroform (100 mL) and water (100 mL). An aliquot of dimethyl sulfate-d$_6$ (1.0 mL, 10.1 mmol) was added and the mixture was poured into a separatory funnel, the layers separated, and the aqueous phase extracted twice with 100 mL of chloroform. The chloroform extracts were combined, dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated by roto-evaporation. The resulting solid was sublimed at reduced pressure (130°C,0.1 mm) to yield 0.9 g of 1-trideuteromethyl-3,7-dimethylxanthine (85%). mp 231°C. IR (KBr pellet) 3100, 2940, 1695, 1650, 1540, 1475, 1450, 1425, 1360, 1280, 1240, 1200, 925, 875, 765, 745, 725, 615 cm^{-1}. ^{1}H NMR (CDCl$_3$) delta 3.60 (s,e) 4.00 (s,3), 7.56 (s,1). Mass spectrum (70 ev) m/e (rel intensity) 55(33), 82(25), 67(30), 109(84), 197, M+(100).

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Preparation of 1,3-Dimethyl-7-trideuteromethylxanthine (24)--

Following the above general procedure, a quantity of theophylline (1.0 g 5.4 mmol), triethylbenzylammonium chloride (0.4 g, 1.8 mmol), sodium hydroxide (0.5 g, 12.5 mmol), and dimethyl sulfate-d_6 (1.0 mL, 10.1 mmol) yielded 0.9 g of 1,3-dimethyl-7-trideuteromethylxanthine (85%). mp 234°C. IR (KBr pellet) 3100, 2940, 1695, 1650, 1600, 1550, 1470, 1450, 1350, 1285, 1235, 1020, 975, 760, 750, 720, 670, 615 cm^{-1}. ^1H NMR ($CDCl_3$) delta 3.36 (s,3), 3.56 (s, 3), 7.50 (s, 1). Mass spectrum (70 ev) m/e (rel intensity) 67(12), 70(16), 58(18), 85(25), 112(87), 197, M+(100).

Preparation of 1,7-Dimethyl-3-trideuteromethylxanthine (23)--

Following the above general procedure, a quantity of 1,7-dimethylxanthine (100 mg, 0.54 mmol), triethylbenzylammonium chloride (0.1 g, 0.4 mmol), sodium hydroxide (0.1 g, 2.5 mmol), and dimethyl sulfate-d_6 (0.2 mL, 2.0 mmol) yielded 71 mg of 1,7-dimethyl-3-trideuteromethylxanthine (67%). mp 231°C. IR (KBr pellet) 3100, 2950, 1695, 1650, 1540, 1470, 1450, 1350, 1275, 1020, 760, 715, 615 cm^{-1}. ^1H NMR ($CDCl_3$) delta 3.40 (s,3), 4.00 (s,3), 7.52 (s,1). Mass spectrum (70 ev) m/e (rel intensity) 68(18), 70(23), 85(27), 57(29), 112(77), 197, M+(100).

Ozonation of 1-Trideuteromethyl-3,7-dimethylxanthine (21)--

Following the above procedure for the ozonation of caffeine, a quantity of 1-trideuteromethyl-3,7-dimethylxanthine (0.2 g, 1.0 mmol) in water (300 mL) was allowed to react with ozone-oxygen. The product mixture was subjected to gas chromatography-mass spectral analysis to yield the following mass spectral data. Compound, retention time (t_r), mass spectrum (70 ev) m/e (rel intensity). Dimethylparabanic acid-d_3, 4.3 min, MS 142, M+ (unlabeled, 2), 129(9), 29(16), 30(18), 57(20), 70(16), 60(21), 28(23), 56(23), 58(25), 59(27), 61(39), 145, M+ (labeled, 100). 1,3-Dimethyl-5-hydroxyhydantoin, 9.8 min, MS, 60(25), 61(37), 91(37), 147, M+ (labeled, 52), 42(54), 45(59), 62(68), 58(75), 59(84), 129(100). 1,3-Dimethyl-5-azabarbituric acid-d_3, 10.1 min, MS 42(7), 44(9), 45(12), 29(16), 70(16), 28(20), 56(21), 30(23), 58(27), 59(32), 61(37), 160, M+ (labeled, 100). 1,3-Dimethyl-5-azauracil-d_3, 11.3 min, 29(12), 55(12), 83(12), 141, M+ (unlabeled, 12), 59(14), 30(18), 84(18), 87(18), 58(20), 42(21), 28(34), 45(54), 56 (57), 144, M+ (labeled, 100). 1,3-Dimethyloxonic acid methylamide-d_3 14.4 min, 29(11), 116(11), 45(14), 57(14), 30(16), 46(18), 28(21), 201, M+(37), 144(46), 173(46), 58(61), 59(75), 87(100).

Ozonation of 1,7-Dimethyl-3-trideuteromethylxanthine (23)--

Following the above procedure for the ozonation of caffeine, a quantity of 1,7-dimethyl-3-trideuteromethylxanthine (50 mg, 0.25 mmol) in water (74 ml) was allowed to react with ozone-oxygen. The product mixture was analyzed by gas chromatography-mass spectrometry (conditions C, rate = 8°C/min) to yield the following mass spectral data, compound, retention time (t_r), mass spectrum (70 ev) m/e (rel intensity). 1,4-Dimethyloxalamide, 4.1 min, MS 119, M+ (labeled, 5), 44(12), 88(12), 59(36), 116, M+ (labeled, 5), 44 (12), 88(12), 59(36), 116, M+ (unlabeled, 54), 58(100). Dimethylparabanic acid-d_3, 5.5 min, MS 117(11), 70(21), 60(25), 142, M+ (Unlabeled, 12), 57(29), 56(30), 59(32), 58(36), 61(45), 145, M+ (Labeled, 100). 1,3-Dimethyl-5-azabarbituric acid-d_3, 11.7 min, MS 130(9), 57(11), 60(11), 56 (16), 58(16), 59(21), 70(21), 61(39), 160, M+(100). 1,3-Dimethyloxonic acid methylamide-d_3, 16.6 min, MS 44(14), 58(23), 201, M+ (labeled, 23), 173 (36), 144(39), 56(64), 83(100).

Ozonation of 1,3-Dimethyl-7-trideuteromethylxanthine (24)--

Following the above general procedure for the ozonation of caffeine, a quantity of 1,3-dimethyl-7-trideuteromethylxanthine (0.2 g, 1.0 mmol) was dissolved in water (300 mL) and allowed to react with ozone-oxygen. The product mixture was subjected to gas chromatography-mass spectral analysis to yield the following mass spectral data: compound, retention time (t_r), mass spectrum (70 ev) m/e (rel intensity). 1,3-Dimethyloxalamide-d_3, 4.0 min, MS 88(9), 44(14), 119, M+ (labeled, 14) 61(29), 59(32), 116, M+ (labeled, 14) 61(20), 59(32), 116, M+ (unlabeled, 45), 58(100). Dimethylparabanic acid, 5.5 min, MS 114(11), 145, M+ (Labeled, 16) 70(18), 57(34), 56(37), 58(59), 142, M+ (unlabeled, 100). 1,3-Dimethyl-5-hydroxyhydantoin 11.3 min, MS 40(9), 30(12), 42(27), 88(27), 144, M+(30). 58(62), 116(64), 59(100). 1,3-Dimethyl-5-azabarbituric acid, 11.7 min, MS 129(9), 128(11), 57(20), 70(21), 56(36), 58(64), 157, M+(100). 1,3-Dimethyl-5-azauracil-d_3, 13.0 min, MS 70(7), 55(11), 69(12), 58(16), 57(18), 86(20), 87(21), 83(29), 42(30), 59(41), 144, M+ (labeled, 41), 56(50), 84(50), 141, M+ (unlabeled, 100). Methylparabanic acid, 13.7 min, MS 42(9), 40(11), 46(16), 42(23), 72 (23), 75(27), 70(46), 57(61), 60(66), 56(71), 100(75), 103(77), 58(80), 128, M+ (unlabeled, 98), 131, M+ (labeled, 100). 1,3-Dimethyloxonic acid methylacide-d_3, 16.2 min, 58(11), 142(12), 173(20), 47(21), 61(21), 141(27), 201, M+ (labeled, 27), 56(57), 83(100).

Preparation of 1,3-Dimethyluracil (25)--

A quantity of uracil (20 g, 178 mmol) and sodium hydroxide (17.0 g, 425 mmol) was dissolved in water (100 mL). The solution was cooled in an ice bath and dimethyl sulfate (53.2 g, 422 mmol) was added. The solution was brought to a boil, cooled to room temperature, and extracted 3 times with chloroform (100 mL). The chloroform extracts were combined, dried over Na_2SO_4, filtered, and concentrated by roto-evaporation. The resulting solid was vacuum dried to yield 22.0 g of 1,3-dimethyluracil (88%). mp 122-124°C. IR (KBr pellet) 1640, 1450, 1330, 1120, 825, 760 cm^{-1}. ^1H NMR ($CDCl_3$) delta 3.32 (s,3), 3.40 (s,3), 5.74 (d,1), 7.18 (d,1). Mass spectrum (70 ev) m/e (rel intensity) 28(12), 42(47), 55(35), 82(18), 83(33), 140, M+(100).

Ozonation of 1,3-Dimethyluracil (25)--

A solution of 1,3-Dimethyluracil (10.0 g, 71 mmol) in water (750 mL) was allowed to react with a stream of ozone-oxygen (600 mL/min, 7 hr). The

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reaction mixture was sparged with N_2 (600 mL/min, 20 min) and the water removed by roto-evaporation. The resulting oil was dissolved in CH_2Cl_2 (100 mL) and passed through a short column of anhydrous Na_2SO_4. The CH_2Cl_2 solution was concentrated to 20 mL volume and subjected to GC analysis (conditions C). The reaction mixture was found to consist of 4 major products (85% of the product mixture). GC data, retention time, (%); Compound A, 3.3 min, (32%); Compound B, 6.1 min, (38%), Compound C, 8.1 min, (11%), Compound D, 11.6 min, (4%). The reaction mixture was chromatographed through silica gel with EtOAc:$CHCl_3$ (80:20) and solvent fractions collected at 20 mL intervals and analyzed by GLC. Compound A was found in fractions 27-32 and Compound B was found in fractions 54-74. Compounds C and D were not resolved as pure components. The respective fractions were concentrated by roto-evaporation to yield 1.5 g of dimethylparabanic acid (A) and 1.0 g of 1,3-dimethyl-5-hydroxhydantoin (B). Dimethylparabanic acid (re- crystallized from EtOH). mp 153°C. IR (KBR pellet) 1720, 1440, 1380, 1090, 750 cm^{-1}. ^{1}H NMR ($CDCl_3$) delta 3.17 (s). Mass spectrum (70 ev) m/e (rel intensity) 27(11), 114(11), 70(19), 29(33), 57(47), 28(51), 56(60), 58(91), 142, M+(100). 1,3-Dimethyl-5-hydroxhydantoin (B): IR (neat) 3350, 2940, 1710, 1485, 1400, 1275, 1225, 1130, 1075, 980, 840, 760 cm^{-1}. ^{1}H NMR ($CDCl_3$) delta 3.02 (s,6), 5.20 (s,1), 5.50 (s,1). Mass spectrum (70 ev) m/e (rel intensity) 15(11), 41(11), 56(11), 29(16), 88(25), 28(26), 144, M+ (26), 30(35), 42(53), 116(54), 58(89), 59(100). Compound C: Mass spectrum (70 ev) m/e (rel intensity) 144(12), 29(14), 56(14), 42(16), 28(21), 59(23), 58(86), 143 M+(100). Compound D: Dimethylparabanic acid, 8.1 min, MS 114 (12), 29(14), 28(20), 70(21), 57(57), 56(61), 142, M+(98), 58(100). 1,3-Dimethyl-5-hydroxy-5-deuterohydantoin, 14.6 min, MS 31(11), 58(21), 89(25), 43(27), 145, M+(36), 59(48), 117(89), 60(100). Mass spectrum (70 ev) m/e (rel intensity) 59(11), 114(12), 70(14), 29(16), 41(16), 42(16), 86(18), 183 (18), 69(21), 28(23), 56(30), 57(35), 227, M+(46), 142(60), 58(88), 143(100).

Preparation of 1,3-Dimethyl-5-deuterouracil (26)--

A quantity of 1,3-dimethyluracil (1.0 g, 7.1 mmol) was dissolved in D_2O (15 g, 0.75 mmol) and 5 drops of $POCl_3$ added. The reaction mixture was refluxed overnight, allowed to cool and extracted 3 times with CH_2Cl_2 (40 mL). The CH_2Cl_2 layers were combined, dried over Na_2SO_4, filtered, and evaporated by roto-evaporation. The solid was sublimed (160°, 0.1 mm) to yield 0.9 g of 1,3-dimethyl-5-deuterouracil (90%). mp 118-120°C. IR (KBr pellet) 2930, 1650, 1440, 1385, 1360, 1335, 1225, 1150, 1020, 945, 860, 780, 680 cm^{-1}. ^{1}H NMR ($CDCl_3$) delta 3.36 (s,3), 3.44 (s,3), 7.27 (s,1). Mass spectrum (70 ev) m/e (rel intensity) 42(11), 83(16), 56(19), 84(29), 141, M+ (100).

Ozonation of 1,3-Dimethyl-5-deerouracil (26)--

Following the procedure for the ozonation of 1,3-dimethyluracil, a quantity of 1,3-dimethyl-5-deuterouracil (0.1 g, 0.71 mmol) in water (5mL) was allowed to react with ozone-oxygen. The product mixture was analyzed

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by gas chromatography-mass spectrometry (conditions A) to yield the following mass spectra. Compound, retention time, Mass spectrum, (70 ev) m/e (rel intensity).

Ozonation of 1,3-Dimethyl-5-hydroxyhydantoin (9)--

Following the procedure for the ozonation of 1,3-dimethyluracil, a quantity of 1,3-dimethyl-5-hydroxyhydantoin (0.1 g, 0.7 mmol) in water (5 mL) was allowed to react with ozone-oxygen. Following workup, the reaction mixture was subjected to gas chromatographic analysis. GC analysis revealed that only starting material, 1,3-dimethyl-5-hydroxyhydantoin, was present in the reaction mixture. $t_r = 11.27$ min., Coinjection with authentic sample, $t_r = 11.36$ min. (conditions B).

Preparation of 1,3,5-Trimethyluracil (28)--

Following the procedure for preparation of 1,3-dimethyluracil (25), a quantity of 5-methyluracil (10.0 g, 79.4 mmol), sodium hydroxide (7.5 g, 189 mmol), and dimethyl sulfate (23.3 g, 185 mmol) yielded 7.4 g (61%) 1,3,5-trimethyluracil (15). mp 135-138°C. ^{1}H NMR (CDCl$_3$) delta 1.94(s,3), 3.34 (s,3), 3.38(s,3), 7.04(s,1). ^{13}C NMR (CDCl$_3$) proton decoupled spectrum, ppm relative to TMS, 13.0, 27.9, 36.6, 109.4, 139.0, 151.9, 164.0 ppm. Mass spectrum (70 ev) m/e (rel intensity) 96(11), 97(12), 42(51), 69(53), 68 (77), 154, M+(100).

Ozonation of 1,3,5-Trimethyluracil (28)--

Following the above conditions for the ozonation of 1,3-dimethyluracil (25), a quantity of 1,3,5-trimethyluracil (1.0 g, 6.5 mmol) was allowed to react with ozone-oxygen (600 mL/min, 17 mg O_3/min) for 45 minutes. The reaction mixture was analyzed by GC-MS (conditions C) to give the following data: Compound, retention time, Mass spectrum (70 ev) m/e (rel intensity), Pyruvic acid methylamide, 1.5 min, MS 15(18), 28(18), 101, M+(21), 43(61), 59(100). 1,3,5-Trimethyl-5-hydroxyhydantoin, 7.2 min, MS 158, M+(11), 31(21), 28(23), 130(25), 30(26), 73(40), 43(54), 56(61), 58(96), 143(100).

ALDEHYDES AND FATTY ACIDS

Aldehyde Ozonation

In 1958 Leffler proposed that the ozonation of an aldehyde was initiated by nucleophilic attack on the carbonyl carbon by ozone.[40] However, Bailey observed that electron donating groups increased the rate of ozonation of aromatic aldehydes[41]. He proposed that the initial attack occurred by way of a 1,3-dipolar insertion mechanism with significant positive charge placed on the carbonyl carbon (see Figure 58). This transition state was proposed to produce a hydrotrioxide intermediate which in turn decomposed to an acid plus singlet oxygen. Low temperature ^{1}H NMR studies conducted by Murray

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substantiate the existence of this hydrotrioxide intermediate. A similar mechanism is proposed for the ozonation of alcohols, amines, and ethers.[36,42,43]

Carboxylic Acid Ozonation

Under normal ozonation conditions carboxylic acids are quite resistant to attack by ozone. Paillard and Brine[44] have shown that if glacial acetic acid is allowed to react with ozone some peracetic acid is produced. Taube[45] determined that the yield of peracetic acid is increased if the reaction is allowed to occur in the presence of ultraviolet light. He proposed that the reaction involved attack of the acid by electronically excited atoms generated from ozone (see Figure 59). Unsaturated fatty acid esters have been shown[11] to yield alkanes, acids, and carbonyl compounds after treatment with ozone followed by hydrolytic, catolytic or pyrolytic decomposition.

The formation of hydrocarbon products upon the ozonation of UTSD wastewater could result from the ozonation of aldehydes, which are identified wastewater contaminants, or alternatively, from the ozonation of carboxylic acids which are aldehyde ozonation products. The formation of hydrocarbons from a peracid could result from decomposition of the peracid into a hydroxyl radical and a carboxyl radical (see Figure 60). The carboxyl radical might then decompose to carbon dioxide and a n-alkyl radical. The n-alkyl radical could abstract a proton from somewhere within the reaction mixture to produce an alkane.

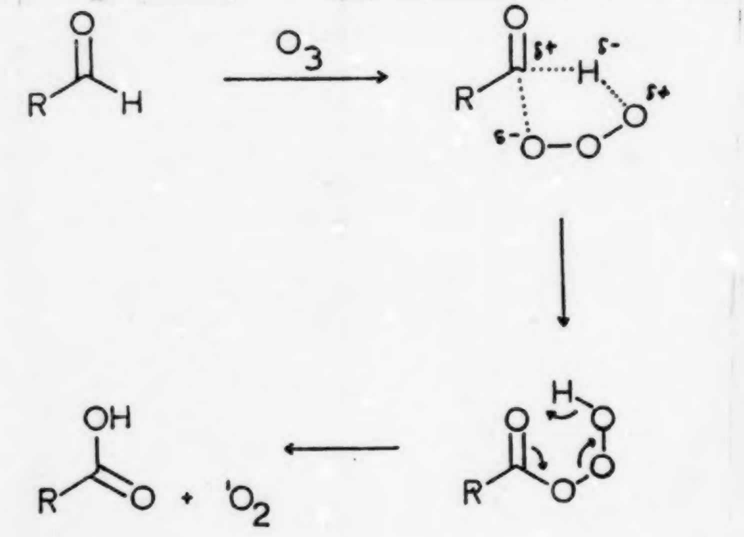

Figure 58. Proposed mechanism for ozonation of an aldehyde.

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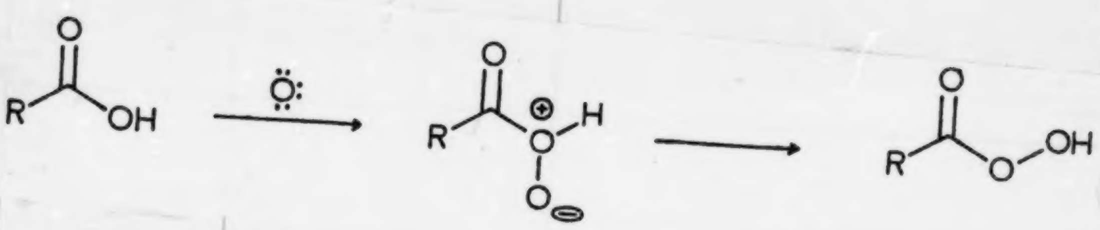

Figure 59. Proposed mechanism for formation of peracetic acid.

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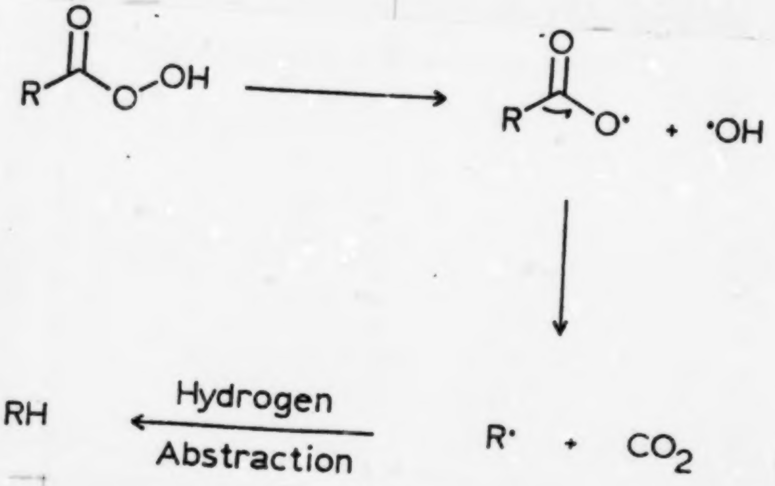
R
OH
·OH
RH
Hydrogen
Abstraction
R·
CO2

Figure 60. Proposed mechanism for hydrocarbon formation from a carboxylic acid.

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The formation of a hydrocarbon from the ozonation of an aldehyde might occur as shown in Figure 61. In this scheme the hydrotrioxide intermediate decomposes to form a hydroxyl radical, singlet oxygen, and a carbonyl radical. The carbonyl radical decomposes with loss of carbon monoxide to yield an alkyl radical. The alkyl radical abstracts a hydrogen atom from within the system to produce an alkane.

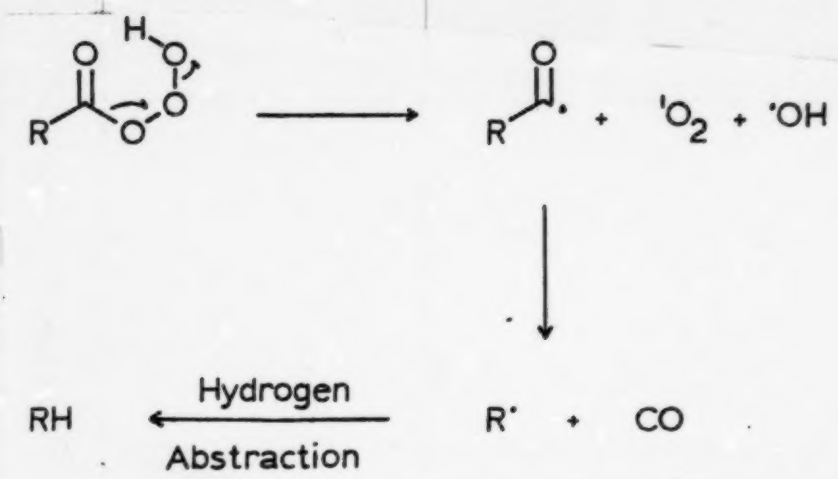

Figure 61. Proposed mechanism for hydrocarbon formation from an aldehyde.

The formation of hydrocarbons during an ozonation reaction has been observed by Greiner and Muller[46] and by Payde, et al [11] after decomposition of isolated reaction intermediates. Greiner and Muller observed that the

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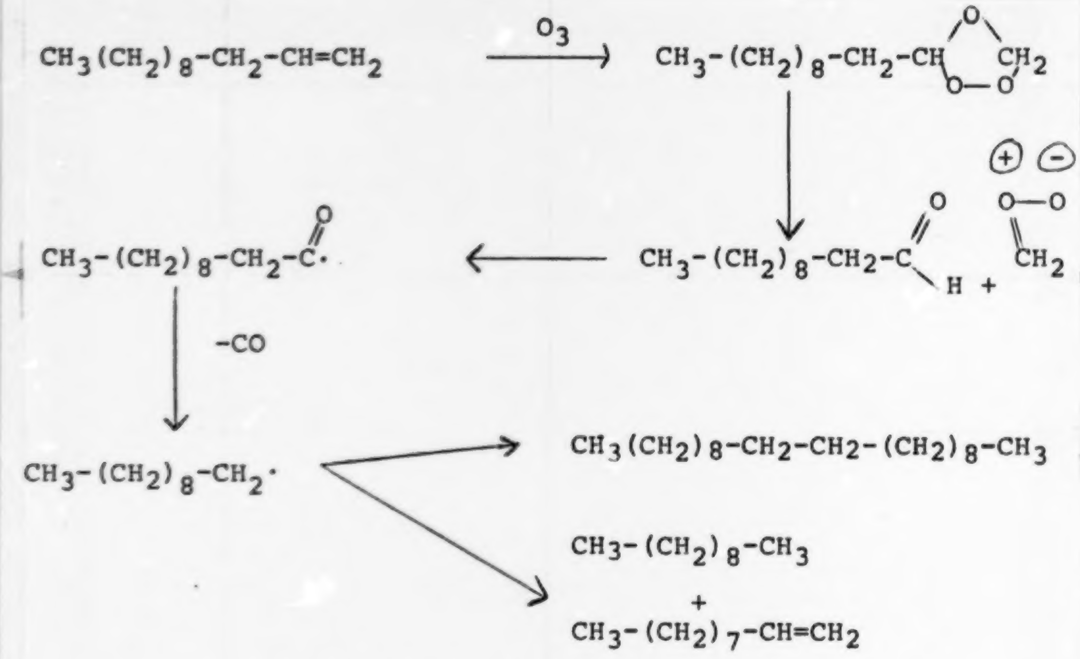
CH3(CH2)8-CH2-CH=CH2
O3
CH3-(CH2)8-CH2-CH
O
CH2
O—O
+
−
O
O—O
CH3-(CH2)8-CH2-C
H +
CH2
O
CH3-(CH2)8-CH2-C·
-CO
CH3-(CH2)8-CH2·
CH3(CH2)8-CH2-CH2-(CH2)8-CH3
CH3-(CH2)8-CH3
+
CH3-(CH2)7-CH=CH2

Figure 62. Proposed mechanism for hydrocarbon formation on an alkene.

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ozonation of 1-docecene occured with the formation of undecanal and a small amount of n-decane. They proposed the mechanism shown in Figure 62 for alkane formation.

Ozonation Experiments

Carboxylic Acids--

The ozonation of dilute aqueous solutions of pelargonic acid, hexanoic benzoic, and octanoic acids was conducted in order to investigate the proposal that hydrocarbon products are produced for carboxylic acids. Hydrocarbon products were not formed and the carboxylic acid was recovered unchanged in each experiment.

Aldehydes--

Aldehydes in dilute aqueous solution were allowed to react with ozone (see Table 16). In all cases hydrocarbon products were formed and represented approximately 1% of the product mixture. The major ozonation product in each case was the respective carboxylic acid. The ozonation of nonanal produces octane and hexandecane. Apparently, hexadecane is formed by the coupling of two n-octyl radicals. In the reaction of hexanal, only the coupling product decane is observed. Pentane, an expected product, is too volatile under the analytical conditions to be detected (see Experimental Section) in these experiments.

Oleic Acid--

The ozonation of the oleic acid, a suspected precursor for nonanal and octane in the Estes Park effluent, was conducted in aqueous solution. The ozonation reaction produced nonandioic acid and nonanal as the major reaction products. In addition, a small amount of nonanoic acid and the hydrocarbons, octane and hexadecane were formed. The results indicate that oleic acid is a likely precursor for octane and nonanal in the ozonized effluent of the UTSD facility. The results from these experiments are analogous to those of Pryde, et al.[11] The reaction products are the stable end-products of ozonation after decomposition of any reactive intermediate species.

Mechanistic Studies

Production of Radical Intermediates--

In order to demonstrate that radical intermediates were formed upon ozonation of aldehydes, a substrate was chosen which was thought to produce relatively stable radical intermediates. Phenylacetaldehyde was allowed to react with ozone and the product mixture analyzed by gas chromatography-mass spectrometry. The data indicated that 1,2-diphenylethane, dibenzylketone, toluene, and benzyl alcohol were formed in the product mixture. The production of these species suggests that the benzyl radical is present as a transient intermediate during the ozonation reaction (see Figure 63). The

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TABLE 16. OZONATION OF ALDEHYDES IN AQUEOUS SOLUTION

Aldehyde	Hydrocarbon Product	Method of Identification GC[1]	Method of Identification GC/MS
Hexanal	Decane	-	X
Octanal	Heptane	X	-
Nonanal	Octane	X	X
	Hexadecane	-	X
Decanal	Nonane	X	X
	Octadecane	-	X
Undecanal	Decane	-	X
Dodecanal	Undecane	X	X
	Docosane	-	X
Hydrocinnam-aldehyde	Ethylbenzene	X	-

[1] Identification by coinjection of authentic sample

[a] Products identified by GC/MS

[b] The major product is the carboxylic acid.

formation of dibenzylketone suggests that the phenylacetyl radical, a proposed precursor for the benzyl radical, is also formed during ozonation.

Hydrogen Abstraction Mechanism--

The work of Greiner and Muller[46] suggests that alkanes are formed by both a coupling mechanism and a disproportionation mechanism (see Figure 62). The disporportionation mechanism involves abstraction of a hydrogen atom by one octyl radical from another octyl radical to generate octane plus octene. The mechanism implies that hydrogen abstraction occurs from the original beta position of the aldehyde. The ozonation of an aldehyde with deuterium atoms attached to the beta carbon might support the validity of this mechanism.

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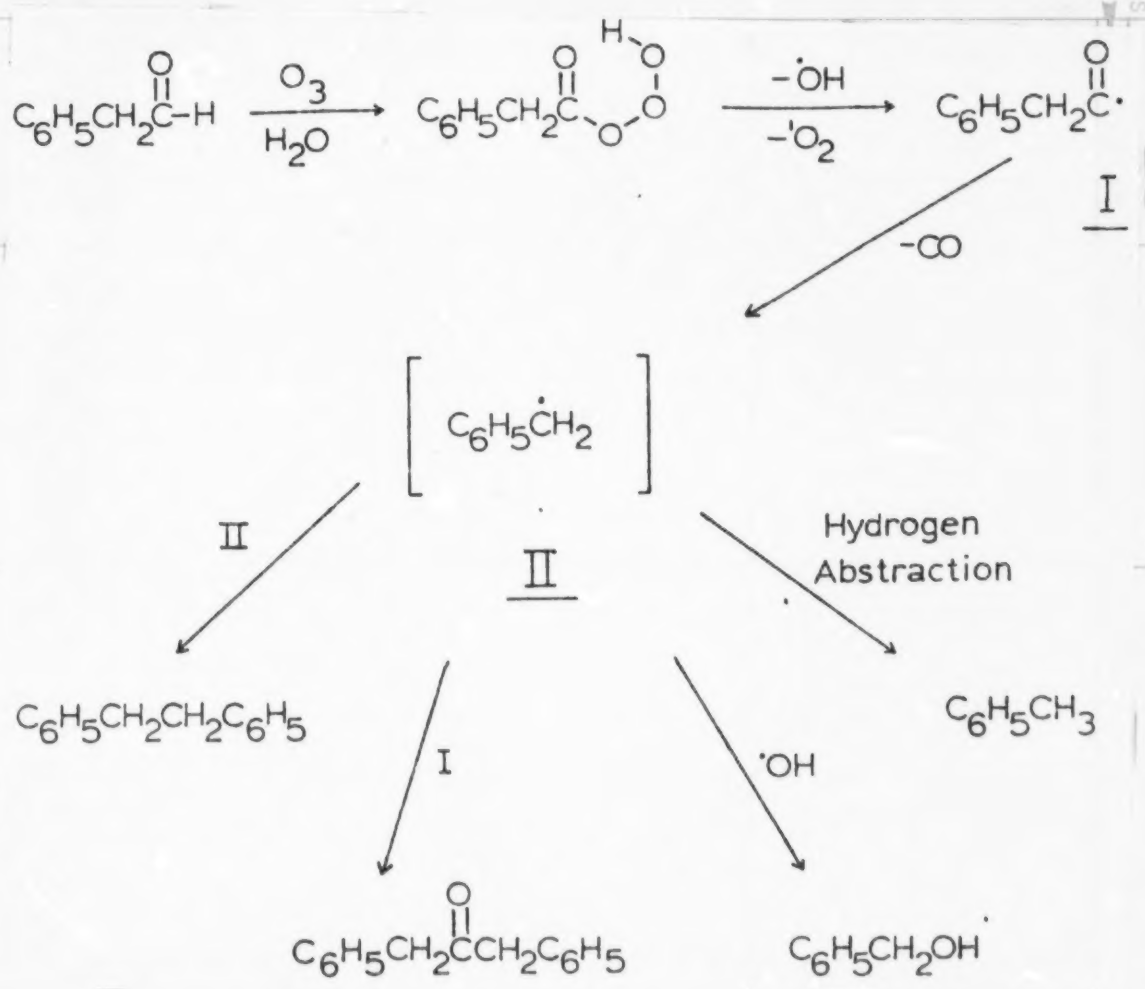
$C_6H_5CH_2C(=O)-H$
O_3
H_2O
$C_6H_5CH_2C(=O)-O-O-O-H$
$-\dot{O}H$
$-{}^{\cdot}O_2$
$C_6H_5CH_2\dot{C}=O$
I
$-CO$
$[C_6H_5\dot{C}H_2]$
II
II
Hydrogen Abstraction
$C_6H_5CH_2CH_2C_6H_5$
I
$\dot{O}H$
$C_6H_5CH_3$
$C_6H_5CH_2C(=O)CH_2C_6H_5$
$C_6H_5CH_2OH$

Figure 63. Ozonation of phenylacetaldehyde.

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The octyl radical could also obtain a hydrogen atom from other sources. The octyl radical could abstract a hydrogen atom from (1) water to generate octane plus a hydroxyl radical, (2) from the carbonyl carbon of the starting aldehyde, (3) from along the hydrocarbon chain randomly and/or from impurities present in the water (see Figure 64). Again, deuterium labeling studies should substantiate the validity of each of these possibilities.

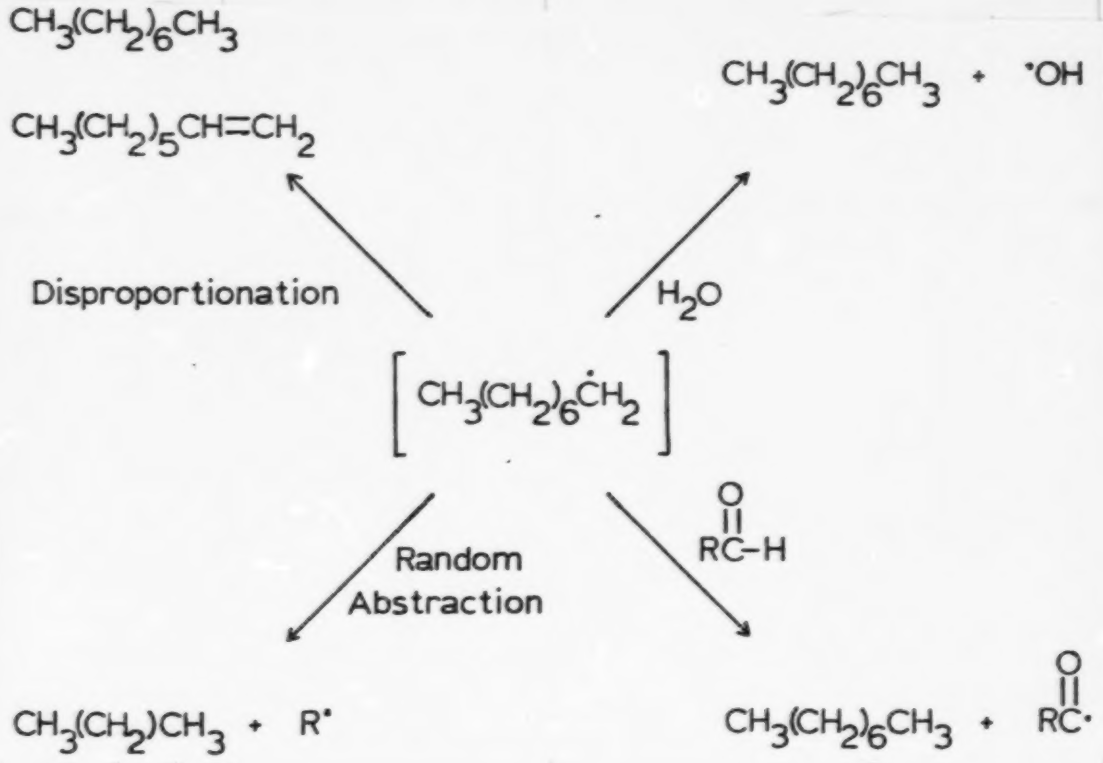
CH3(CH2)6CH3
CH3(CH2)5CH=CH2
CH3(CH2)6CH3 + •OH
Disproportionation
H2O
CH3(CH2)6ĊH2
Random Abstraction
RC(=O)–H
CH3(CH2)CH3 + R•
CH3(CH2)6CH3 + RC(=O)•

Figure 64. Proposed reactions of the octyl radical.

The ozonation of nonanal and oleic acid was conducted in deuterium oxide solution. In each case octane is produced. However, this material was

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not found to be enriched with a deuterium content greater than that expected from natural abundance. This result demonstrates that the octyl radical does not abstract a proton from water to produce octane.

The proposed abstraction of a hydrogen atom from the carbonyl carbon of the aldehyde was examined with the 1-deuterio labeled aldehydes. Nonanal-d_1 and undecanal-d_1 were prepared by treating the respective carboxylic acids with lithium aluminum deuteride to form the 1,1-dideutero alcohols. The alcohols were oxidized with chromium trioxide-pyridine to produce the 1-deuterio aldehydes. Each aldehyde was ozonated in pure water and the resulting alkane analyzed by GC/MS for deuterium incorporation. Deuterium incorporation was not observed in either case and this indicates that the alkyl radical does not abstract a hydrogen atom from the carbonyl carbon of the aldehyde.

The preparation of 3,3-dideuterioundecanal was accomplished by the scheme shown in Figure 65. The ozonation of 3,3-dideuterundecanal produced 2,2-dideuteriodecane. The hydrocarbon did not contain a significant enrichment by a third deuterium atom as predicted by the disproportionation mechanism (see Figure 66). The disproportionation mechanism does not appear to be involved in hydrocarbon formation.

The hydrogen abstraction mechanism for hydrocarbon formation remains to be ascertained. There is no question that ozonation of an aliphatic aldehyde or an unsaturated fatty acid results in the hydrocarbon formation. The experimental data suggests that alkanes are formed by radical intermediates rather than ionic intermediates. The data also suggest that these radicals abstract hydrogen atoms from reactants, products or trace impurities by a random process.

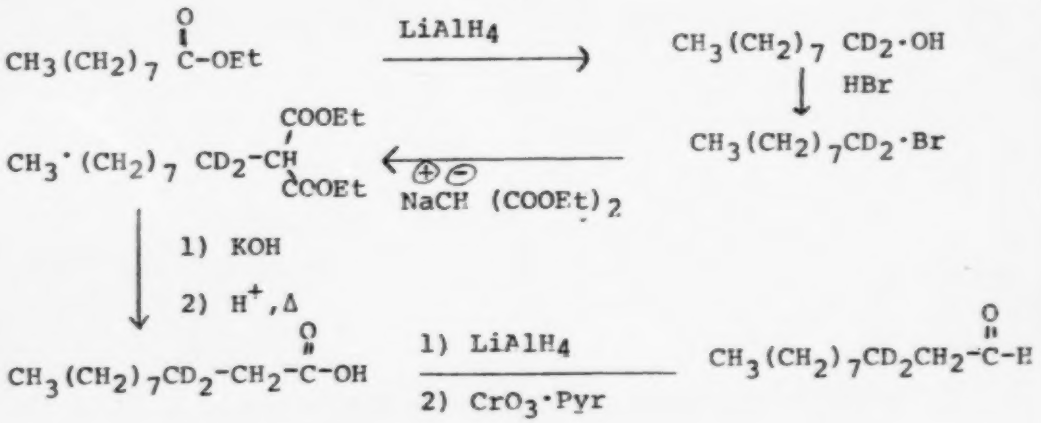

Figure 65. Preparation of 3,3 - Dideuteroundecanal.

$$2\ RCH_2CH_2\cdot \longrightarrow RCH{=}CH_2 + RCH_2CH_3$$

Figure 66. Disproportionation of alkyl radical.

Experimental

^1H NMR spectra were obtained on a Varian EM-390 90 MHz instrument (chemical shifts are expressed in ppm relative to TMS). Mass spectra were obtained on a Hewlett-Packard 5982A GC/MS system. 1-Bromo-1,1-dideuterionanane was prepared from 1,1-dideuteriononanol according to the procedure of Kamm and Marvel[47]. 3,3-Dideuterioundecanoic acid was prepared from 1-bromo-1,1-dideuteriononane according to the procedure of Reid and Ruhoff.[48] Unlabeled starting materials were purchased from commercial sources.

1,1-Dideuteriononanol (I)--

The reaction was conducted in a 100 mL 2-neck flask fitted with a reflux condensor, addition funnel and a drying tube. Nonanoic acid (2.0 g, 12.7 mmol) in 20 mL anhydrous ether was added dropwise to a magnetically stirred solution of lithium aluminum hydride (0.4 g, 10.3 mmol) in 40 mL anhydrous ether. The solution was stirred at room temperature for 1 hour. Water (0.4 g), sodium hydroxide (0.4 g, 15% solution), and water (1.2 g) were added. The solution was stirred for 15 minutes, and filtered to remove aluminum salts. The ether was removed at reduced pressure and the alcohol (1 g, 55% yield was distilled at 116°C/16mm.

1,1-Dideuterioundecanol--

1,1-Dideuterioundecanol was prepared from the corresponding carboxylic acid by the previous method. The product was distilled at reduced pressure (74-6°C/0.19 mm) to give 1.3 g (69%) product. The ^1H NMR ($CDCl_3$) absorption at 3.6 ppm expected from the alphahydrogens of the corresponding alcohol was absent.

1-Deuteriononanal (II)--

1,1-Dideuteriononanol (1.0 g, 6.9 mmol) in 5 mL dichloromethane was rapidly added to a magnetically stirred solution of pyridinium chlorochromate (2.3 g, 10.7 mmol). After two hours at room temperature the solution was diluted with ether, the solvent decanted, and the black residue washed twice with ether. The extracts were filtered through Florisil (60-100 mesh) and the solvent evaporated at reduced pressure. The oil was distilled at reduced pressure (46°C/0.2 mm) to give 0.2 g (20%) product. Mass spectrum (70 ev) m/z (rel. intensity) 125 (5), 115 (14), 98 (70), 55 (71), 70 (76), 57 (77), 56 (82), 41 (100).

1-Deuterioundecanal--

1-Deuterioundecanal was prepared in the same manner as 1-deuteriononanal. 1,1-dideuterioundecanol (1.2 g, 6.9 mmol) was allowed to react

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with pyridinium chlorochromate (2.3 g, 10.7 mmol) to yield 0.6 g, 1-deuterioundecanal (51%, bp 45°C/0.05 mm). The ^1H NMR absorbance at 9.8 ppm, assigned to a hydrogen atom attached to the carbonyl carbon of an aldehyde, was absent. Mass spectrum (70 ev) m/z (rel. intensity) 153 (1), 143 (7), 126 (35), 58 (64), 56 (67), 57 (68), 83 (86), 69 (90), 43 (100).

Ethyl Nonanoate--

The ester was made by the method of Allen and Kalm.[49] Nonanoic acid (20 g, 0.11 mmol) and concentrated sulfuric acid (2 mL) were added to 200 mL of absolute ethanol. The mixture was shaken momentarily and allowed to stand for 4 hours. The mixture was diluted with water and extracted twice with petroleum ether. The extracts were combined, dried over anhydrous Na_2SO_4, filtered and the solvent removed by rotoevaporation. The product (16 g, 60% yield) was distilled at 90°C/10 mm.

3,3-Dideuterioundecanol--

2,2-Dideuterioundecanoic acid (2.3 g, 12.2 mmol) was allowed to react with lithium aluminum hydride by the method used to prepare 1,1-dideuteriononanol. The product (2.0 g, 44% yield) was distilled at 131°C/15 mm.

3,3-Dideuterioundecanal was synthesized from 3,3-dideuterioundecanol (1.7 g, 9.8 mmol), was allowed to react with pyridinium chlorochromate (3.3 g, 15.2 mmol) to yield 1.0 g (59%) 3,3-dideuterioundecanal. The aldehyde was distilled at 45°C/0.05 mm. ^1H NMR ($CDCl_3$) 0.90 (M,3), 1.30 (M,14), 2.45 (s,2), 9.88 (t,1). Mass spectrum (70 ev) m/z (rel. intensity) 142 (8), 128 (33), 83 (63), 71 (71), 70 (72), 69 (74), 84 (100).

General Procedure For Ozonation Reactions--

The following parameters were employed for ozonation reactions, unless stated otherwise. The reactions were conducted in a 1000 mL 3-neck round-bottom flask. The ozone-oxygen stream was introduced into the reaction mixture by a gas dispersion tube. The substrate (8 mmol) in water (750 mL) was allowed to react with an ozone-oxygen flow of 300 mL/min. The reaction was magnetically stirred and allowed to proceed for 1 hour. CAUTION: The literature reports at least one violent explosion for a reaction similar to those carried out in these studies.[11]

General Work-Up Procedure A--

The ozonated solution was extracted with 50 mL ether, the extract dried over anhydrous sodium sulfate, and concentrated to 0.5 mL in a Kuderna-Danish vessel. The concentrated sample was subjected to GC/MS analysis.

General Work-Up Procedure B--

Upon completion of the ozonation reaction, excess ozone was destroyed with 1 g sodium thiosulfate. The volatile compounds were purged onto a water cooled Tenax trap with a nitrogen flow of 150 mL/min. The nitrogen flow was allowed to proceed for 2 hours. The temperature of the solution was brought to 70° C during the first hour and then maintained at 70° C for an additional hour. Water vapor was removed from the Tenax trap at room temperature by a nitrogen flow (150 mL/min, 30 min). The analysis was performed by heat desorption (250° C) of the volatile components onto a Hewlett-Packard Model 5982A GC/MS system.

Ozonation of Hexanal--

GC analysis was conducted on an OV-17 glass column under the following conditions: Temp. 1, 65°C; time 1, 0 min; rate 10°C/min; temp. 2, 200°C; time 2, 5 min; attenuation 2^7, N_2 flow, 20 mL/min. The GC analysis showed one major product with retention time 5.25 min. The compound was shown to be hexanoic acid by comparison with an authentic sample (t_r = 5.23 min).

GC/MS analysis demonstrated the presence of a hydrocarbon product and gave the following data; Mass spectrum (70 ev) m/z (rel. intensity) 142 (5), 41 (27), 70 (31), 85 (33), 58 (38), 71 (49), 57 (84), 43 (100). The product was assigned the structure of m-decane. This assignment was substantiated by mass spectral comparison with an authentic sample of n-decane. The results shown in Table 16 were obtained in a similar fashion. In all cases product identifications were established by GC/MS comparison with authentic samples.

Ozonation of Nonanal In Deuterium Oxide (Work-Up A)--

Nonanal (25 g, 1.8 mmol) was placed in a 50 mL 3-neck round-bottom flask containing D_2O (25 mL) and allowed to react with an ozone-oxygen stream (150 mL/min) for 30 minutes. GC/MS analysis was conducted on a glass-OV-1 column under the following conditions: Temp 1, 70°C; time 1, 2 min; rate 16°C/min; Temp 2, 250°C. The analysis showed a peak with rt 1.1 min. which gave the following mass spectrum: m/z (rel. intensity) 115 (1), 114 (13), 70 (25), 56 (30), 71 (39), 41 (44), 57 (48), 85 (66), 43 (100). The compound was identified as n-octane. The product was not enriched in deuterium content.

Ozonation of Oleic Acid In Deuterium Oxide (Work-Up A)--

Oleic acid (25 g, 0.09 mmol) was allowed to react with an ozone-oxygen stream as described in the previous experiment. GC/MS analysis (as in previous experiment) showed the presence of a product with retention time 1.1 min which gave the following mass spectrum m/z (rel. intensity) 115 (1), 114 (8), 29 (18), 56 (23), 71 (32), 41 (40), 57 (42), 85 (48), 43 (100). The product was identified as n-octane and was not enriched in deuterium content.

Ozonation of 1-Deuteriononanal (Work-Up A)--

1-Deuteriononanal (15 mg) was allowed to react in H_2O (1.5 mL) with an ozone-oxygen stream. GC/MS analysis (as in previous experiment) showed a peak with retention time 1.0 min., which gave the following mass spectrum: m/z (rel. intensity) 115 (3), 114 (23), 70 (31), 56 (39), 41 (48), 71 (52), 57 (57), 85 (94), 43 (100). The product was identified as n-octane and was not enriched in deuterium content.

Ozonation of 1-Deuterioundecanal In Deuterium Oxide (Work-Up A)--

1-Deuterioundecanal (50 mg., 0.29 mmol) in D_2O (25 mL) was allowed to react with an ozone-oxygen flow (150 mL/min) for 1 hour. The GC/MS analysis was conducted on a 8 ft. Ultra Bond glass column under the following conditions (Temp 1, 50° C, time 1,1 min, rate 15°C/min, Temp 2, 250°C). The analysis showed a product with retention time 2.2 min. which gave the following mass spectrum m/z (rel. intensity) 143 (0), 142 (12), 55 (23), 70 (36), 85 (36), 71 (58), 41 (73), 43 (85), 57 (100). The product was identified as n-decane and was not found to be enriched in deuterium content.

Ozonation of 3,3-Dideuterioundecanal (Work-Up A)--

3,3-Dideuterioundecanal was allowed to react with ozone-oxygen and product analysis conducted under the conditions established in the previous experiment. GC/MS analysis showed a product with retention time 2.2 min which gave the following mass spectrum (rel. intensity) m/z 145 (5), 144 (18), 85 (41), 56 (46), 45 (55), 71 (57), 43 (90), 41 (95), 57 (100). The product was identified as 2,2-dideuteriodecane. The product did not contain isotopic enrichment by a third deuterium atom.

LIGNOSITE® DEGRADATION BY OZONATION

The aqueous ozonation of calcium lignin sulfonate (Lignosite®) was chosen as a model system for our studies of a paper mill wastewater stream. This model was chosen because approximately 50% of the spent sulfite liquor solids is lignin.[50]

Lignosite® is a refined form of calcium lignin sulfonate which is derived from spent sulfite liquor, a by-product of the acid-sulfite wood treatment process in pulp mills. The sulfite liquor solids are stripped several times to remove sulfur dioxide, while fermentable hexose sugars are removed by alcoholic fermentation. The refined calcium sulfonate is marketed as a dry, light brown powder by the Georgia Pacific Corporation under the trade name Lignosite^R. This product typically contains 80% calcium lignin sulfonates, 15% carbohydrates and carbohydrate sulfonates and 5% minor constituents.[51] The calcium lignin sulfonates are polymers with molecular ranges from 20,000 to 70,000 and structures similar to Figure 67 on the next page[52].

Aqueous solutions of Lignosite® were either left acidic (unbuffered) or made alkaline ($KHCO_3$), then reacted with ozone and analyzed by solvent

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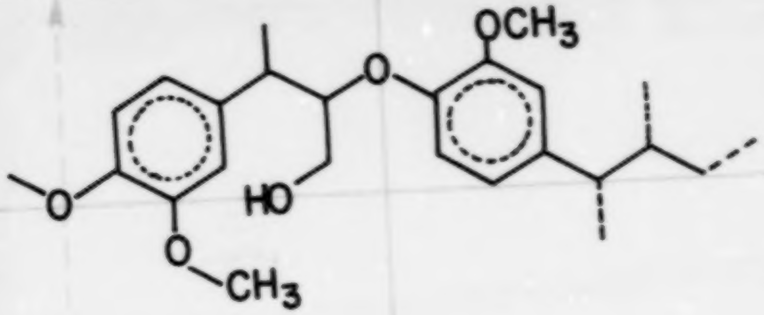

Figure 67. Structure of a model repeating unit for calcium lignin sulfonate.

extraction followed by GC/MS/DS. The organic soluble products of the acidic ozonation included vanillin and homovanillic acid (see Figure 68). The organic soluble products of the alkaline reaction included toluene, m-xylene, phenol, a C_4-substituted benzene and several C_3-substituted benzenes as well as vanillin and homovanillic acid.

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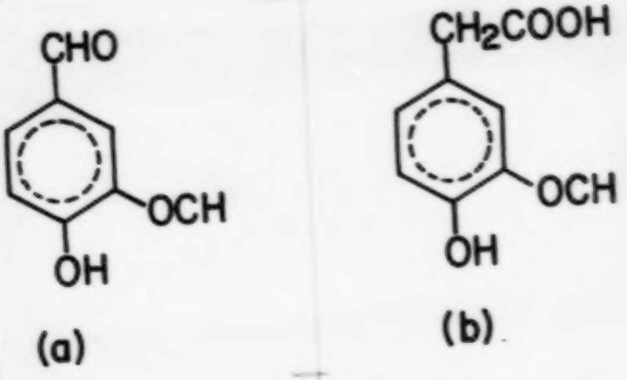

Figure 68. Structures of vanillin (A) and homovanillic acid (B).

Experimental

Acidic Aqueous Ozonation--

Lignosite® powder (5 g) and distilled water (500 mL) were placed in a flask (750 mL, one-neck) fitted with an ozone dispersion tube, an ozone outlet and a magnetic stirring bar (Teflon®). The mixture was stirred, yielding a dark brown solution, then treated with a stream of ozone in oxygen (4% w/w, 1 mL s^{-1}, 1/2 hour).

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The reaction mixture was extracted with chloroform and the organic layer separated, dried (Na_2SO_4) and concentrated for analysis by GC/MS/DS.

Basic Aqueous Ozonation--

A solution of Lignosite® in distilled water (10%) was prepared as above then brought to pH 8 ($KHCO_3$). The solution was then treated with a stream of ozone-oxygen (4%, 2-3 mL s^{-1}, 1 hour).

The reaction mixture was then acidified and extracted with chloroform. The organic layer was separated, dried (Na_2SO_4) and concentrated for analysis by GC/MS/DS.

MISCELLANEOUS INDUSTRIAL CONTAMINANTS

p-Chlorophenyl Methylsulfone

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The ozonation of p-chlorophenylmethyl sulfone was conducted in a 14-liter stainless steel reactor equipped with mechanical stirrer, quartz immersion wells, and sample withdrawel capability. A quantity of p-chlorophenylmethyl sulfone (1.0 g) was dissolved in 10 liters of water and allowed to react with an ozone-oxygen stream under ultraviolet irradiation. Aliquots (300 mL) of the reaction mixture were withdrawn at regular intervals (15 min). The aliquots were extracted with CH_2CL_2, the CH_2CL_2 layers dried (Na_2SO_4), and concentrated by roto-evaporation. A known quantity of a standard (naphthalene) was added to the concentrate and the resulting mixture analyzed by gas chromatography. In a second experiment, an identical solution of p-chlorophenyl sulfone was ozonated in the presence of filtered (Vycor®) ultraviolet irradiation. The results of each of these experiments are shown in Table 17 and Figure 69. The data indicate that the rate of disappearance of sulfone is greater when the ultraviolet light is not filtered by a Vycor filter. A plot of reaction time versus the natural logarithm of sulfone concentration (Figure 69) indicates that the reaction is first order with respect to sulfone concentration in each case. The rate of reaction in the presence of unfiltered ultraviolet irradiation ($k=3.8 \times 10^{-2}$ min^{-1}) is approximately four times faster than that conducted in the presence of filtered ultraviolet irradiation ($k=1.0 \times 10^{-2}$ min^{-1}).

Diisopropylmethylphosphonate (DIMP)

The photochemical ozonation of diisopropylmethylphosphonate (DIMP) was conducted in a stainless steel reactor equipped with two quartz immersion wells. Unfiltered ultraviolet irradiation was obtained from a Pen Ray lamp purchased from Ultraviolet Products, Inc., San Gabriel, California. The reaction was conducted at an oxygen-ozone flow of 3.7 liters/minute (1.83-3.7% ozone).

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The ozonation reaction was performed using a 10 L solution containing 400 ppm DIMP in water. The progress of the reaction was monitored by gas chromatography and shown to be complete after 6 hours. When 1 L of the aqueous reaction mixture was concentrated to dryness an oily substance remained. This material was dissolved in DMSO-d_6:$CDCl_3$ and the proton magnetic resonance (PMR) spectrum obtained. This PMR spectrum is similar to that of methylphosphonic acid. The doublet centered at 1.40 ppm is attributed to a methyl group bonded to phosphorus. The singlet at 7.12 ppm is apparently the result of exchange of the acidic methylphosphonic acid protons with residual water in the sample. A mass spectrum of this sample was obtained and is shown in Figure 70. The mass spectrum was found to be identical to that obtained on an authetic sample of methylphosphonic acid (see Figure 71).

These results establish that DIMP is converted under the reaction conditions to methylphosphonic acid(see Figure 72). Further studies are in progress to determine the fate of the isopropyl portion of the DIMP molecule.

Dicyclopentadiene

The ozonation of dicyclopentadiene was conducted in the presence and absence of ultraviolet irradiation. In both cases the reaction was conducted in a 3-necked glass reaction vessel. The ultraviolet irradiation was introduced through a water cooled quartz immersion well and was produced from a Hannovia 450 watt medium pressure mercury lamp. In each reaction 20 g of dicyclopentadiene was slurried in 750 mL of water and allowed to react for 4 hours with an oxygen-ozone stream (600 mL/min, 17 mg O_3/min). The results of each reaction appear to be similar at least in a qualitative sense.

Each reaction was spontaneous upon ozone introduction and produced a white aerosol or smoke. This aerosol was difficult to condense but could be removed partially from the gas stream upon passage through a cold finger (-78°C). This aerosol formation was concurrent with precipitation of a white solid from the reaction mixture. This white precipitate was collected and dried. A mass spectrum of the precipitate (see Figure 73) suggested the presence of a substance which had increased in molecular weight by 32 mass units (Mol wt 164). The proposed structure for this substance is shown in Figure 74 and is that of either structure 1 or 3. This material was found to be relatively insoluble in common organic solvents ($CHCl_3$, CH_2Cl_2, CH_3OH, DMSO) and subsequent PMR analysis was not successful. A 5.0 g quantity of this material was subjected to reduction by excess $LiAlH_4$. GC/MS analysis indicated that several new substances were produced (Figure 75). An unidentified substance having molecular weight 220 (retention time 3.7 min) was produced (Figure 76). Two components which elute at 8.3 and 9.0 min appear to be isomeric and give weak or non-existent molecular ion at 168 mass units (see Figures 77 and 78). Each compound shows a M-18 mass loss which is apparently due to loss of water from the molecular ion. The new ion (m/z 150)

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TABLE 17 RATE OF REACTION OF P-CHLOROPHENYLMETHYL SULFONE WITH OZONE-ULTRAVIOLET LIGHT

Reaction With Vicor Filter			Reaction Without Vicor Filter		
Time(min)	(Sulfone), (ppm)	In(Sulfone)	Time(min)	(Sulfone), (ppm)	In(Sulfone)
0	98.4	4.59	0	98.4	4.59
15	74.7	4.31	15	28.1	3.34
30	64.3	4.16	30	17.2	2.84
45	57.7	4.06	45	12.6	2.53
60	49.8	3.91	60	7.7	2.04
75	42.5	3.75	75	5.6	1.72
90	41.5	3.73	90	3.2	1.16
105	33.6	3.51	105	1.3	0.26
120	27.0	3.30	120	0.6	-0.51
135	23.8	3.17	135	0.4	-0.92
150	19.0	2.94	150		

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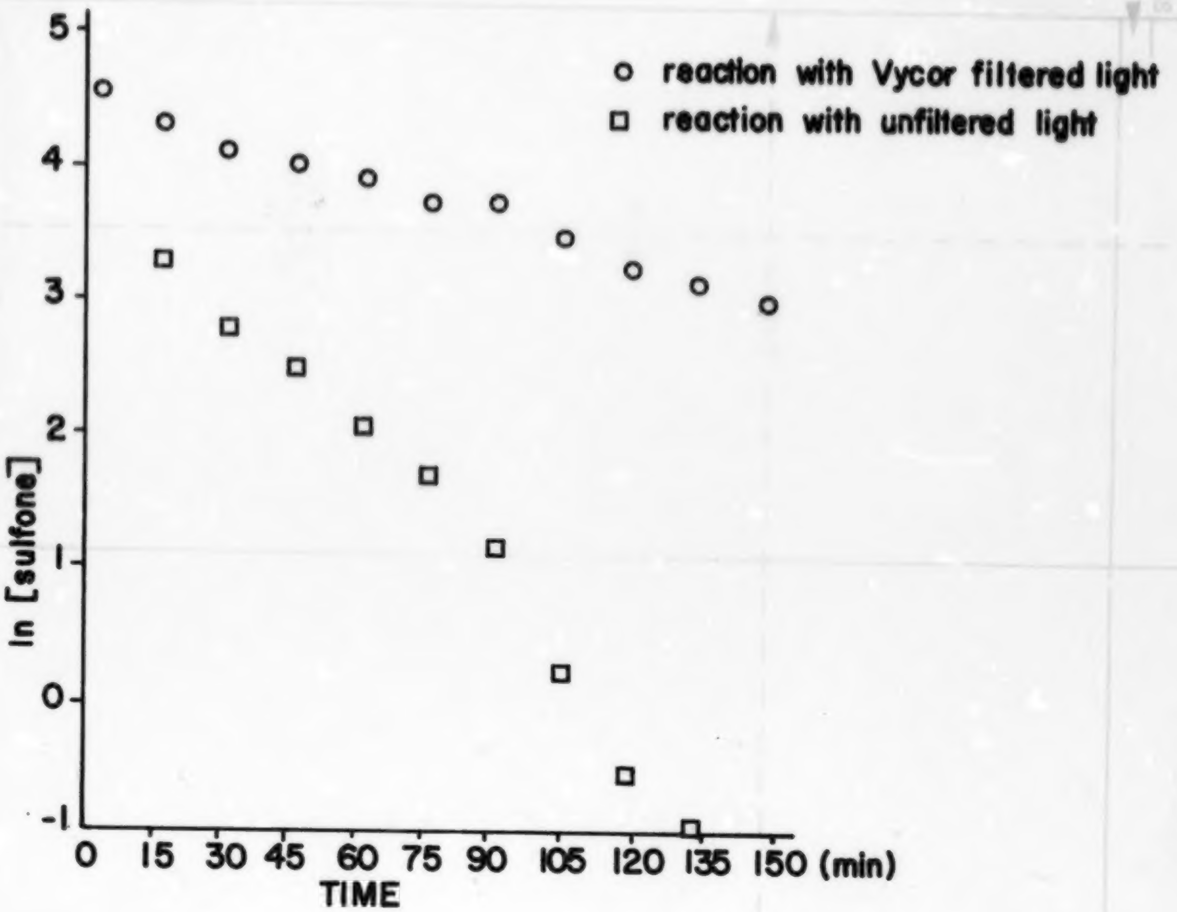

Figure 69. Kinetics of ozonation of p-chlorophenylmethyl sulfone under ultraviolet irradiation with and without Vycor filter.

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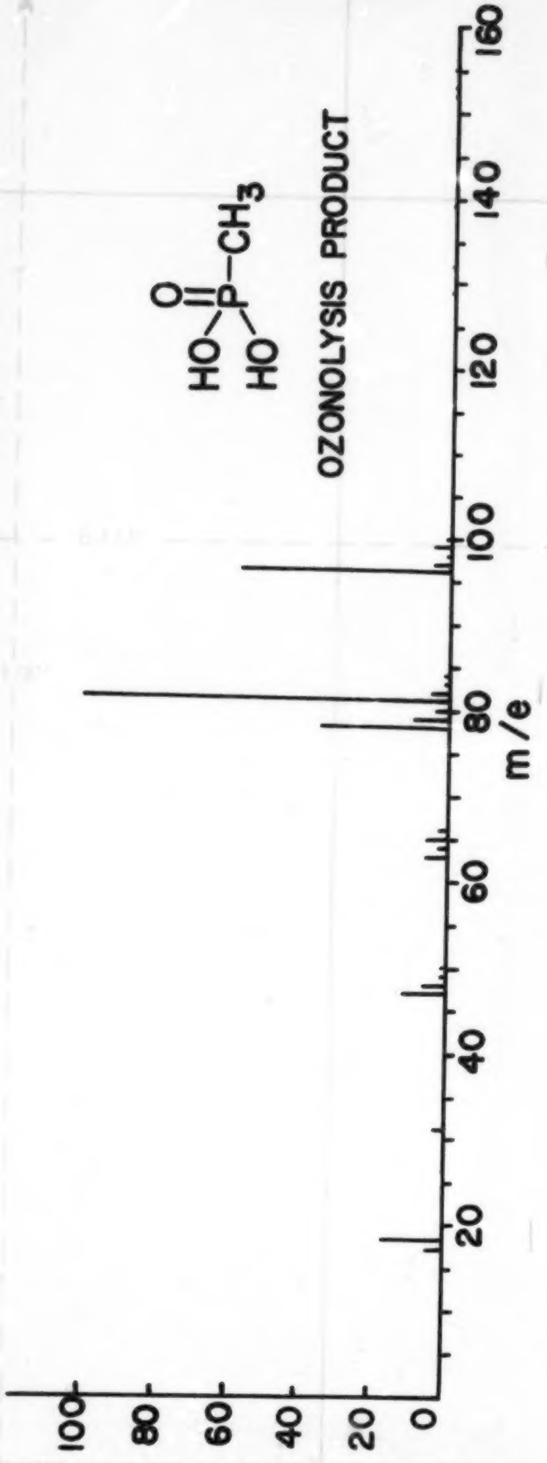

Figure 70. Mass spectrum of diisopropylmethylphosphonate (DIMP) ozonolysis product.

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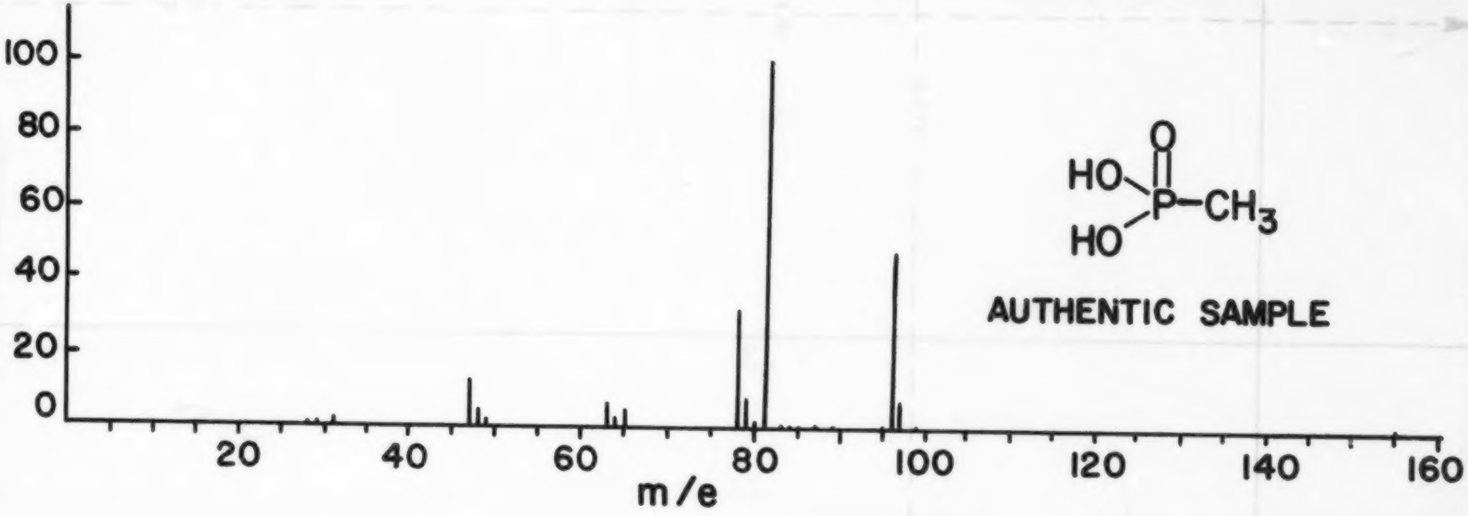

Figure 71. Mass spectrum of methylphosphonic acid.

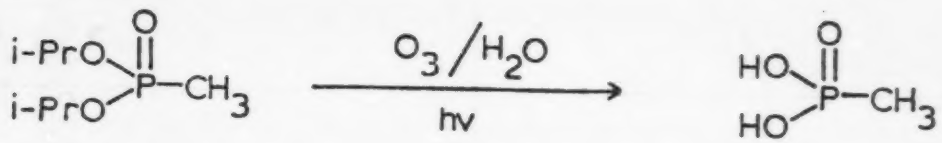

Figure 72. Ozonation of DIMP.

apparently undergoes further loss of 31 mass units to give the base peak in the spectrum (m/z 119). Each of these fragmentation processes is indicative of that shown by a primary alcohol. Also present in each of these mass spectra are ions of significant intensity at m/z 66. On the basis of this data these reduction products are proposed to have either structure 2 or 4 shown in Figure 74. The isomeric nature of these compounds is thought to be the result of the presence of cis and trans dicyclopentadiene in the starting material in which the trans form is predominant (trans refers to the orientation in which the cyclopentane ring is bonded to the endo positions of the bicyclic ring). The proposal that structure 2 is the correct structure is supported by the fact that a significant ion at m/z 66 is found in the mass spectrum of each isomer. This fragment ion is apparently the result of a retro Diels-Alder reaction which produces cyclopentadiene plus a neutral fragment in the mass spectrometer. The mass spectrum of dicyclopentadiene shows this type of fragmentation with m/z = 66 as the base peak in the spectrum (see Figure 79). This type of fragmentation is expected for structures of type 2 but not for those of type 4. The proposal that structure 2 is not the correct structure is suggested by the fact that addition to the dicyclopentadiene ring system usually occurs at the 5,6 double bond and not the 2,3 double bond.[53] If ozone behaves in the same manner, structure 4 is the correct structure. It should be noted that this information is speculative and awaits further confirmation.

Dicyclopentadiene (low concentration)

A solution of dicyclopentadiene (17.2 ppm) in water was ozonated in the presence of ultraviolet irradiation. A gaseous aerosol was formed but a solid precipitate did not form (a solid precipitate is formed when dicyclopentadiene is ozonated as a slurry in water, i.e., at high concentration). The destruction of DCPD versus time was determined (see Table 18). The results demonstrate the DCPD concentration was reduced by greater than 99% after a 90 minute ozonation.

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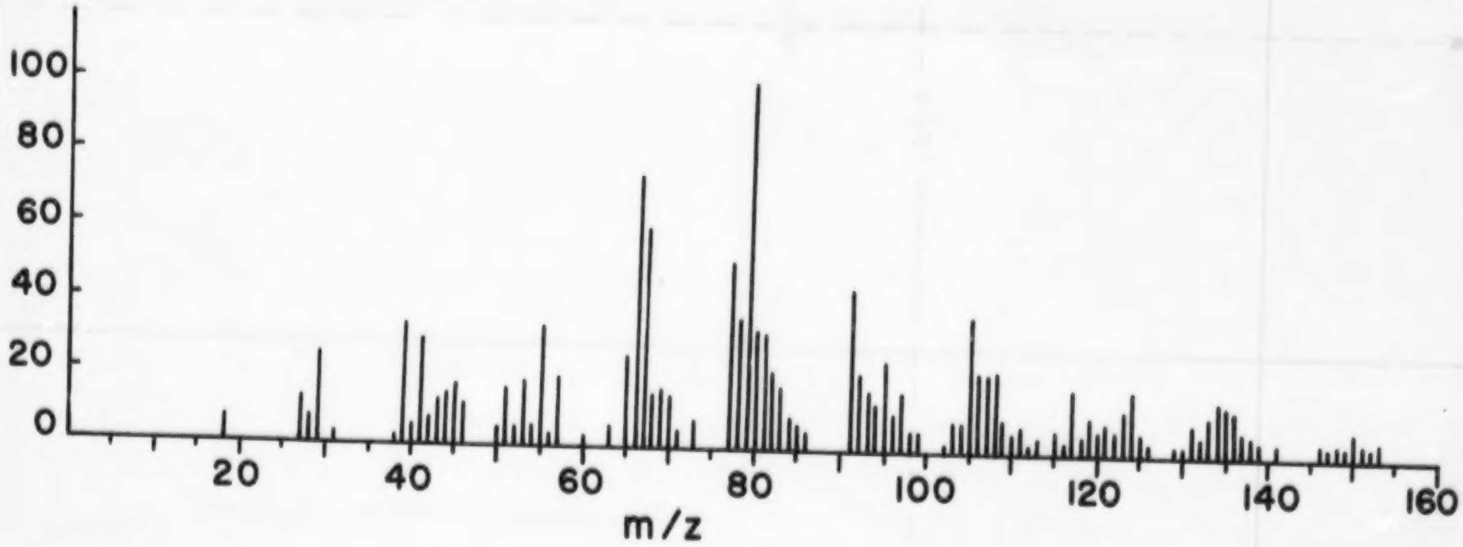

Figure 73. Mass spectrum of precipitate from dicyclopentadiene ozonolysis.

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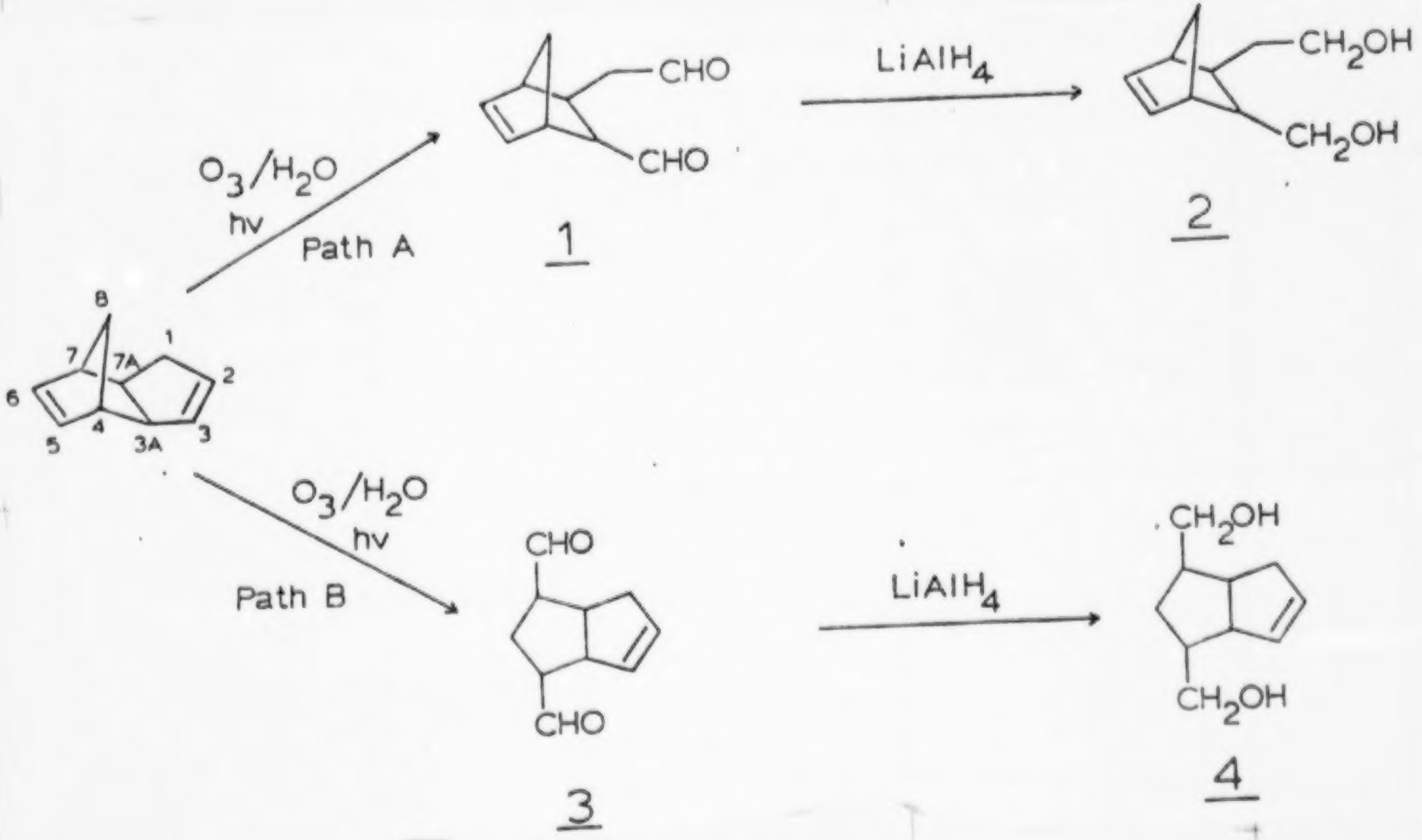
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hv
Path A
O$_3$/H$_2$O
hv
Path B
8
7
7A
1
2
6
5
4
3A
3
CHO
CHO
1
LiAlH$_4$
CH$_2$OH
CH$_2$OH
2
CHO
CHO
3
LiAlH$_4$
CH$_2$OH
CH$_2$OH
4

Figure 74. Ozonolysis of dicyclopentadiene.

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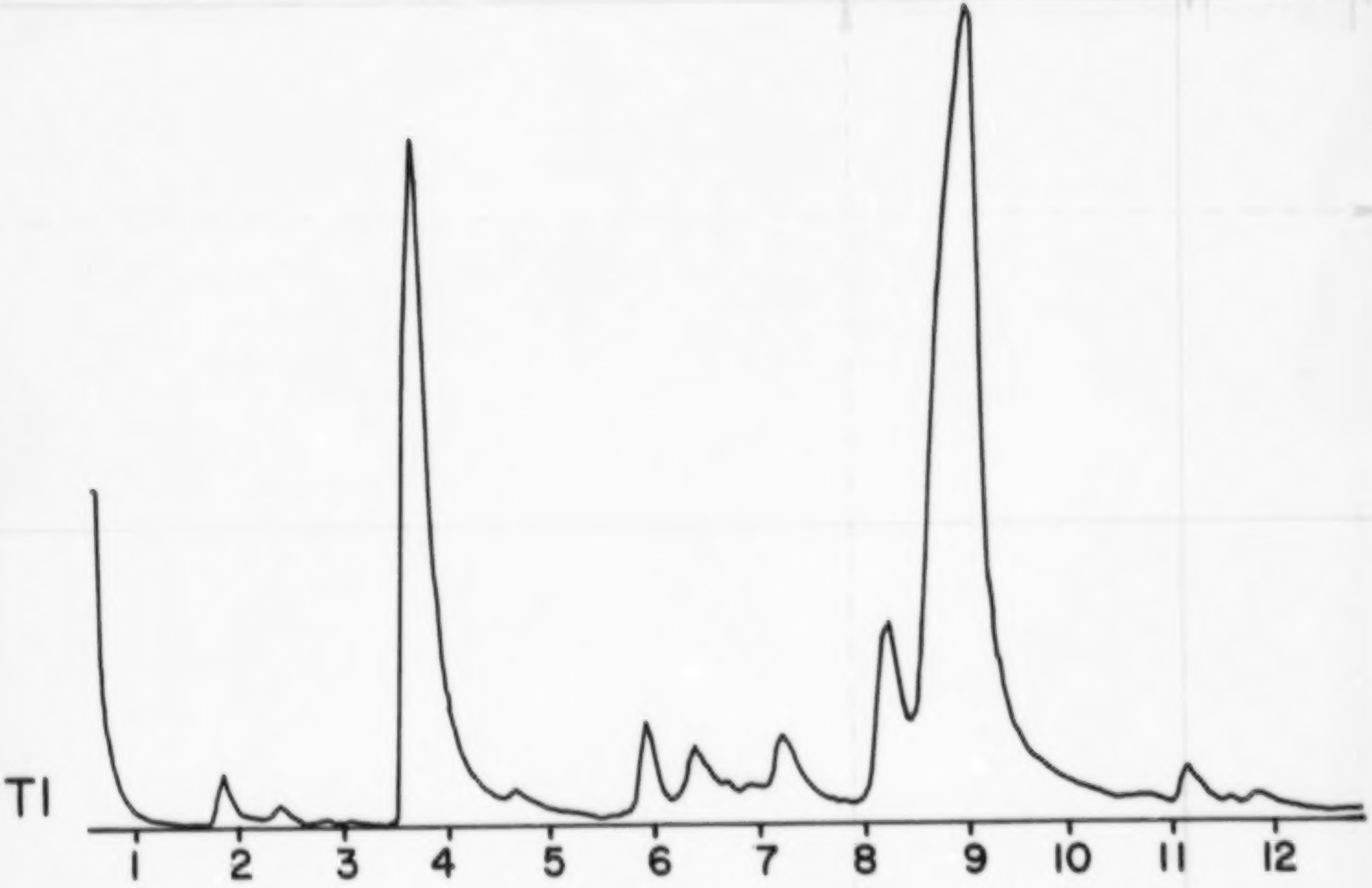

Figure 75. Total ion chromatogram of $LiAlH_4$ reduction products.

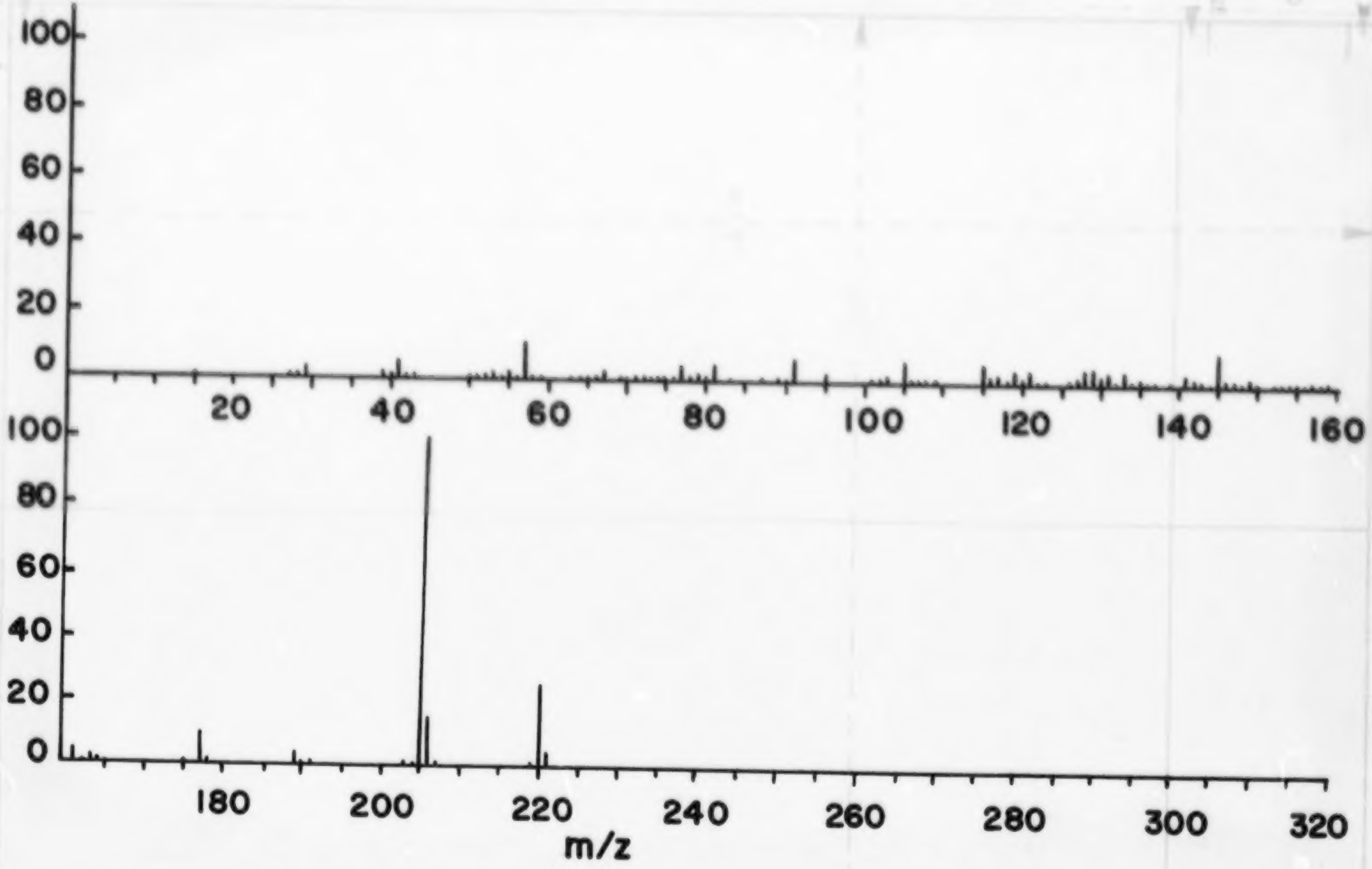

Figure 76. Unknown substance formed during $LiAlH_4$ reduction.

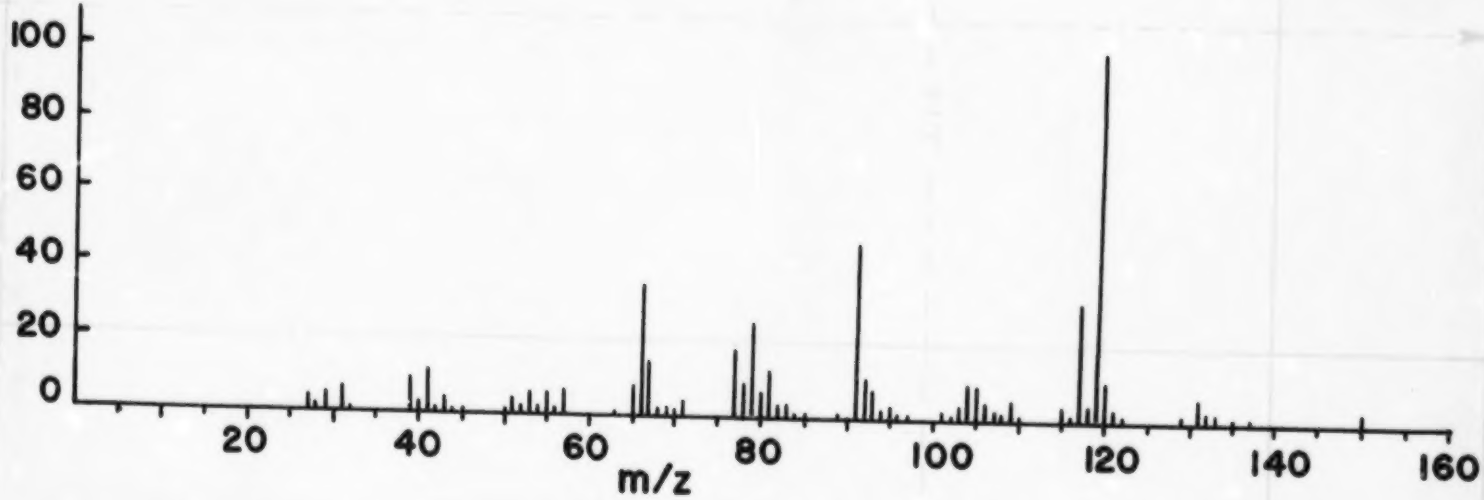

Figure 77. $LiAlH_4$ reduction product (A).

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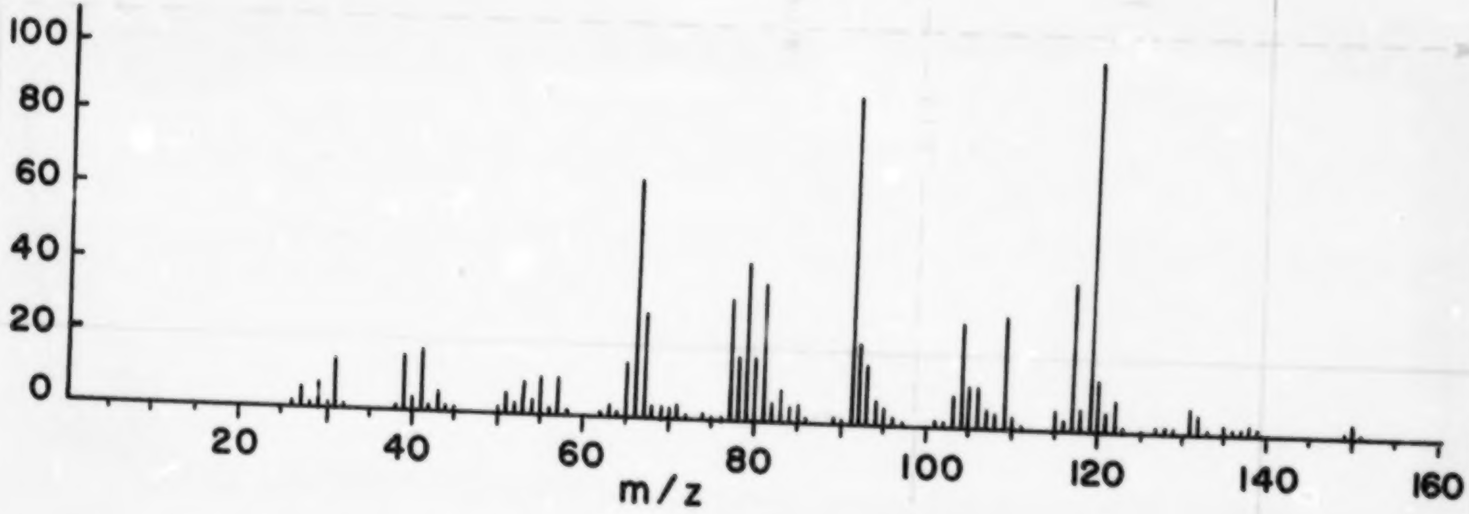

Figure 78. $LiAlH_4$ reduction product (B).

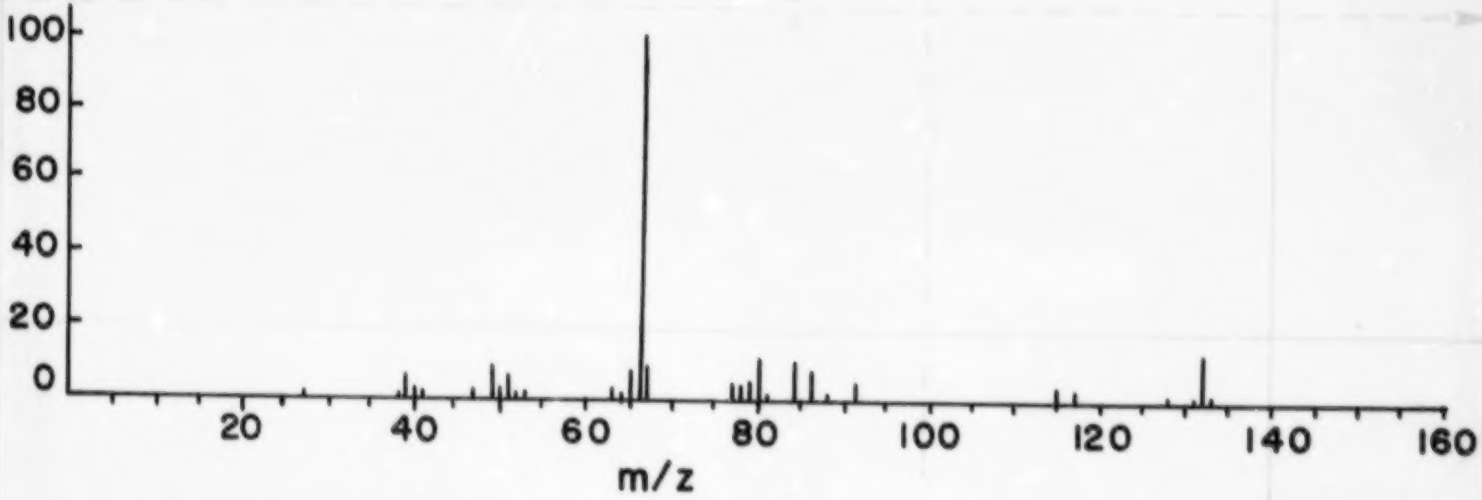

Figure 79. Mass spectrum of dicyclopentadiene.

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TABLE 18. DESTRUCTION OF DICYCLOPENTADIENE[a]

TIME	CONCENTRATION
Initial	17.2 ppm
15 Minutes	734 ppb
45 Minutes	255 ppb
60 Minutes	187 ppb
90 Minutes	<30 ppb

---

[a] Results obtained by gas chromatography internal standard method.

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